FORMULATION, DEVELOPMENT AND *IN VITRO* CHARACTERIZATION OF CANDESARTAN CILEXETIL MUCOADHESIVE MICROBEADS

Dissertation submitted to

THE TAMIL NADU Dr. M. G. R. MEDICAL UNIVERSITY

CHENNAI-32

In partial fulfillment of the requirement for the

Degree of

MASTER OF PHARMACY IN PHARMACEUTICS

Submitted By

(Reg.No-26108609)

APRIL 2012

DEPARTMENT OF PHARMACEUTICS
COLLEGE OF PHARMACY
MADURAI MEDICAL COLLEGE
MADURAI – 625 020

Dr.AJITHADAS ARUNA M.Pharm., Ph.D,
Principal,
College of Pharmacy,
Madurai Medical College,
Madurai– 625020.

CERTIFICATE

This is to certify that the dissertation entitled**, "FORMULATION, DEVELOPMENT AND *IN VITRO* CHARACTERIZATION OF CANDESARTAN CILEXETIL MUCOADHESIVE MICROBEADS"** Submitted by **Mr. J.VARUN** in the Department of Pharmaceutics, Madurai Medical College, Madurai – 20, in partial fulfillment of the requirement for the Degree of **Master of Pharmacy** in **Pharmaceutics**, is a bonafide work carried out by him, under the guidance and supervision of **Prof. Mr.A.Abdul Hasan Sathali**, **M.Pharm.,(Ph.D)** Professor and Head, in the Department of Pharmaceutics, Madurai Medical College, Madurai-20,during the academic year 2011 – 2012.

This dissertation is forwarded to the Controller of Examinations, The Tamilnadu Dr. M.G.R. Medical University, Chennai.

Station: Madurai **(AJITHADAS ARUNA)**

Date :

Prof. Mr. A. Abdul Hasan Sathali, M.Pharm.,(Ph.D)
Professor and Head,
Department of Pharmaceutics,
College of Pharmacy,
Madurai Medical College,
Madurai-625 020

CERTIFICATE

This is to certify that the Dissertation entitled **"FORMULATION, DEVELOPMENT AND *IN VITRO* CHARACTERIZATION OF CANDESARTAN CILEXETIL MUCOADHESIVE MICROBEADS"** submitted by **Mr.J.VARUN** in partial fulfillment of the requirement for the degree of **Master of Pharmacy** in **Pharmaceutics** is a bonafide work carried out by him under my guidance and supervision during the academic year 2011 – 2012 in the Department of Pharmaceutics, Madurai Medical College, Madurai-20.

I wish him success in all his endeavors.

Place: Madurai

Date: **(Prof. Mr.A.Abdul Hasan Sathali)**

ACKNOWLEDGEMENT

It is my pleasure to express my respectful regards and thanks to ***Mr.Dr.A.Edwin Joe*** *M.D., F.M., B.L., Dean, Madurai Medical College, Madurai for providing all kinds of supportive facilities required to carry out my project work.*

It is my privilege to extend my gratitude to **Dr. Ajithadas Aruna, M.Pharm., Ph.D.,** *Principal, College of pharmacy, Madurai Medical College, Madurai for her support to carry out my project work.*

It is my immense pleasure and honour to express my deep sense of gratitude and heartfelt thanks to ***Prof. Mr. A. Abdul Hasan Sathali, M.Pharm.,(Ph.D).,*** *Head and Professor, Department of Pharmaceutics for his excellence in guidance, contribution and encouragement which helped me in the successful completion of each and every stage of my project work.*

With immense pleasure I record here my indebtness and hearty thanks to ***Mr. C. Pandian, M.Pharm., Mrs. D. Uma Maheswari, M.Pharm.,*** *and* ***Mr. R. Senthil prabhu, M.Pharm****., Department of pharmaceutics for his support and valuable suggestions throuhtout my work.*

I also extend my thanks to our department staffs ***Mrs. Mumtaj, Mrs. Geetha*** *and* ***Mrs.Chitravalli*** *for their contribution throughout my project work.*

I express my heartiest thanks to ***Ranbaxy Laboratories, Gurgaon*** *for providing the drug candesartan cilexetil as gift sample and* ***United Scientifics*** *and* ***universal drug & chemical suppliers*** *for providing chemicals to carry out my project work.*

I take this privilege to convey my thanks to ***Mr.Vincent sagayaraj M.Sc., Technical officer St Joshep's College, Madurai*** *for his helping to carry out* ***FT - IR*** *studies in accordance with my dissertation work.*

I convey my sincere thanks to ***Mr. K. Gowthamarajan M.Pharm., Ph.D****.,* ***J.S.S College of pharmacy, Ooty*** *for their help in carrying out the* ***DSC*** *studies in accordance with my dissertation work.*

I am very much thankful to ***Mrs.Lavanya Anbu****, Pharma Information Centre, Chennai, for her help in reference collections regarding my project.*

I wish to express my heartiest thanks to my seniors ***Mr.R.Anbhazagan, Mr.R.Jeyasuresh, Mr.M. Muthuramalingam*** *and* ***Mrs.G. Magudeswari.***

Also I would like to extend my sincere thanks to my seniors, ***Ms.A.Gokila, Mrs.R.Kavitha, Ms.K.Priyanka, Ms.P.Shanmugapriya and Ms.T.Sangeetha*** *for their moral support.*

I would like to give my sincere thanks to my friends ***Mr S.Ganesan, Mr.S.Kathiravan, Mr.V.Palanivel, Mr.T.Prakash, Mr.D.RajivGandhi, Ms.R.Revathi, Mr.V.Selvaraj, Ms.T.Suganya, Ms.B.Yuganya*** *for their timely help and co-operation.*

I would like to give my sincere thanks to my juniors ***Ms. C. Deepa., Ms. M. Gomathi., Mr. M. Gopinath., Mrs. J. Jayalakshmi., Mr. L. Magesh kumar., Mr. P.Mainkandan., Mr. I. Samdurai., Ms N.Surya devi., Ms. V.Susila devi., & Ms. N. Nisha.,*** *for their timely help and co-operation.*

I also extend my thanks to all the staff members and P.G. Students of Department of Pharmaceutical Chemistry and Pharmacognosy for their Co-operation.

I honestly acknowledge the love, care and moral support rendered by my family members & friends whose part cannot be expressed in holophrastic.

I am extremely thankful to the staffs of Star Xerox, City Xerox and ***Laser Point,*** *for their kind co-operation regarding printing and binding of this dissertation work.*

CONTENTS

CHAPTER – I

INTRODUCTION

CONTROLLED DRUG DELIVERY SYSTEM

For many decades treatment of an acute disease or a chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical dosage forms including tablets, capsules, suppositories, creams, ointments, aerosols, injectables etc. But historically, oral drug administration has been the predominant route of administration for drug delivery.

Conventional drug delivery systems are the primary pharmaceutical products commonly seen in the prescription and over the counter drug market. But, conventional drug delivery system achieves as well as maintains the drug concentration within the therapeutically effective range needed for treatment only when taken several times a day. This results in a significant fluctuation in drug levels.

There are several terms used interchangeably viz. controlled release, programmed release, sustained release, prolonged release, timed release, slow release, extended release and other such dosage forms. However, controlled release differs from sustained release systems which simply prolong the drug release and hence plasma drug levels for an extended period of time (i.e. not necessarily at a predetermined rate). Thus, the chief objective should be controlled delivery of drugs to reduce dosing frequency to an extent that once daily dose is sufficient for therapeutic management through a uniform plasma concentration at steady state as shown in Fig

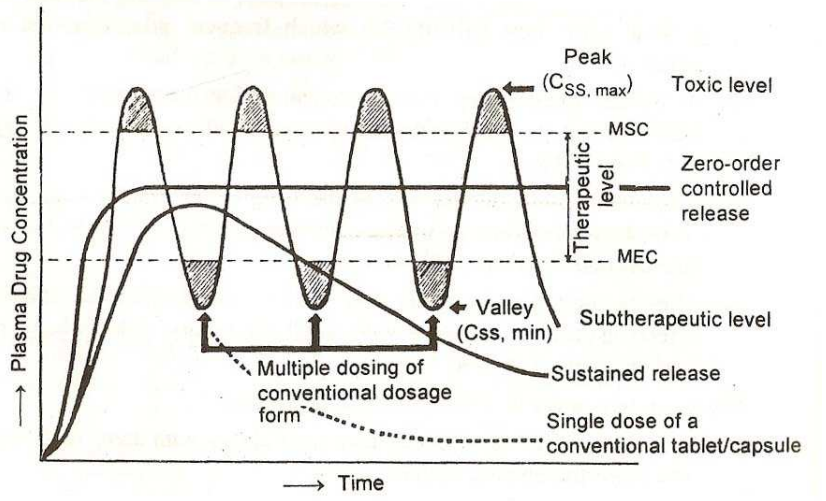


Fig: A hypothetical plasma concentration-time profile

Oral drug delivery is the most widely utilized route of administration among all the routes that have been explored for the systemic delivery of drugs via various pharmaceutical products of different dosage forms. The reasons that the oral route achieved such popularity may be in part attributed to its ease of administration as well as least aseptic constraints and flexibility in the design of the dosage form.

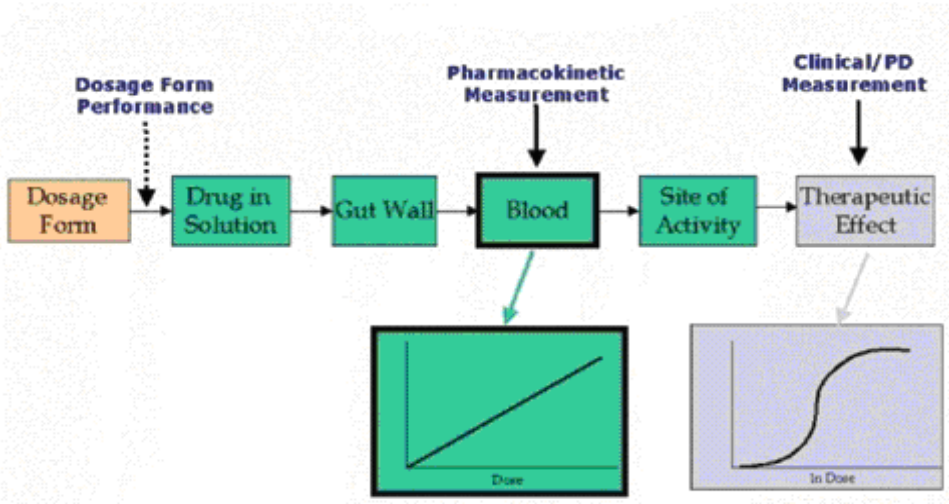


Fig: Model of Oral Dosage Form Performance

The objective of any drug delivery system is to release promptly a therapeutic amount of drug at the site of administration, and then to maintain the desired therapeutic drug concentration at the site of action that elicits the desired pharmacological action, also it minimizes the incidence and the severity of unwanted adverse effects. An appropriately designed for extended / sustained release dosage form can be a major advancement in this direction.

The performance of a drug presented as a controlled release system depends upon its:

- Release from the formulation
- Movement within the body during its passage to the site of action

The former depends upon the fabrication of the formulation and the physiochemical properties of the drug while the latter element is dependent upon pharmacokinetics of drug. In comparison to conventional dosage form where the rate-limiting step in drug availability is usually absorption through the bio-membrane, whereas the rate-determining step in the availability of a drug from controlled delivery system is the rate of release of drug from the dosage form which is much smaller than the intrinsic absorption rate of the drug. The rate limiting step in the design of controlled drug delivery system is shown in Fig.

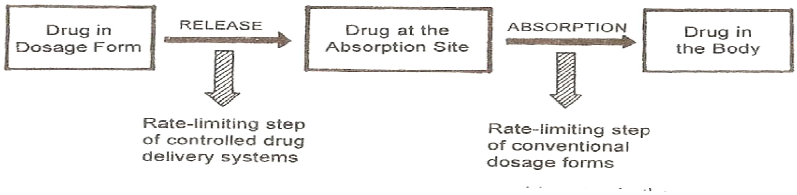


Fig: Scheme representing the rate-limiting step in the design of controlled drug delivery system

Therapeutic advantages of Extended Release dosage forms include,

- reduction in the frequency of drug administration
- improved patient compliance
- maintenance of drug level in blood without oscillations
- reduction in total amount of drug administered
- maximum availability with minimum dose
- improved treatment of many chronic illness
- reduction in health care costs through improved therapy, shorter treatment period, less frequency of dosing and reduction in personnel time to dispense, administer and monitor patients.

During past two decades, numerous oral delivery systems have been developed to act as drug reservoirs from which the active substance can be released over a defined period of time at a predetermined and controlled rate. From a pharmacokinetic point of view, the ideal sustained and controlled release dosage form should be comparable with an intravenous infusion, which supplies continuously the amount of drug needed to maintain constant plasma levels once the steady state is reached.

The most important objectives of the New Drug Delivery Systems are to

i) be a single dose

ii) reduce the duration of treatment

iii) release the active ingredient over an extended period of time

iv) deliver the active entity directly to the site of action

v) minimizing or eliminating side effects.

The controlled release systems for oral use are mostly solids and based on

dissolution, diffusion or a combination of both mechanisms in the control of release rate of drug. Depending upon the manner of drug release, these systems are classified as follows:

A. Continuous Release Systems: These systems release the drug for a prolonged period of time along the entire length of GIT (especially upto the terminal region of small intestine) with normal transit of the dosage form. The various systems under this category are:

1. Dissolution controlled release systems
2. Diffusion controlled release systems
3. Dissolution and diffusion controlled release systems
4. Ion-exchange resin-drug complexes
5. Slow dissolving salts and complexes
6. pH dependent formulations
7. Osmotic pressure controlled systems
8. Hydrodynamic pressure controlled systems

B. Delayed Transit and Continuous Release Systems: These systems are designed to prolong their residence in the GIT along with their release. Often, the dosage form is fabricated to detain the stomach and hence the drug present therein should be stable to gastric pH. Systems included in this category are:

1. Altered density systems
2. Muco-adhesive systems
3. Size-based systems

C.Delayed Release Systems: The design of such systems involve release of drug only at

a specific site in the GIT. The drugs contained such a system are those that are destroyed in the stomach or by intestinal enzymes, or known to cause gastric distress, or absorbed from a specific intestinal site, or meant to exert local effect at a specific GI site.

The two types of delayed release systems are:

1. Intestinal release systems
2. Colonic release systems

In recent years scientific and technological advancements have also been made in the research and development of rate-controlled oral drug delivery systems by over-coming physiological adversities, such as short gastric residence times (GRT) and unpredictable gastric emptying times (GET). Gastro retentive dosage forms have been designed to overcome various difficulties of conventional oral dosage forms.

Several technical approaches are currently utilized in the prolongation of the GRT, including floating drug delivery systems (FDDS), also known as hydro dynamically balanced systems (HBS), swelling and expanding systems, polymeric bio-adhesive systems, muco-adhesive systems, modified-shape systems, high-density systems, magnetic systems, raft forming and other delayed gastric emptying devices.

Gastro retentive drug delivery systems have been shown to have better efficacy in controlling the release rate of drugs with site-specific absorption. Bioadhesive, super porous hydrogel, floating and expanding systems shows the most promising potential for achieving the goal of gastric retention. From the formulation and technological point of view, the floating drug delivery system is considerably easy and logical approach.

Gastric retentive dosage forms have been investigated to provide controlled release therapy for drugs with reduced absorption in the lower gastro intestinal (GI) tract

or for local treatment of diseases of the stomach or upper GI tract. Gastric retentive dosage forms rely on either natural GI physiology, such as floating or large tablets that depend on delayed emptying from the fed stomach, or those dosage forms that are designed to fight the physiology and avoid emptying in the fasted state through dosage forms of larger sizes with or without flotation or bioadhesion.

Oral administration of a medication by means of controlled drug delivery systems should ideally enable to obtain the required plasma concentration and to maintain the steady state level for a prolonged period of time.

Many drugs categorized as once-a-day delivery have been demonstrated to have sub-optimal absorption due to dependence on the transit time of the dosage form, making traditional extended release development challenging. Therefore, a system designed for longer gastric retention will extend the time, within which drug absorption can occur in the gastro intestinal tract.

Since the Gastric Emptying Time is from 2 to 6 hours in humans under fed state, a sustained release dosage form administered orally does not reach sufficient bioavailability and so prolongation of the effective plasma level is not obtained occasionally.

These physiological limitations could be overcome, for various judiciously selected drugs, by prolonging the gastric residence time of the pharmaceutical dosage form. Various approaches have been followed to encourage gastric retention of an oral dosage form.

The different approaches proposed to prolong the residence time of delivery systems in the GIT are:

• the use of floating drug delivery system that have low bulk density so that they can float on the gastric juice in the stomach and release the drug in sustained manner.

• the use of passage-delaying excipients (for example triethanolamine myristate)

• the utilization of specially designed dosage forms such as 'heavy pellets' and large single-unit delivery systems

• bioadhesive or mucoadhesive systems containing bio/mucoadhesive agents, enabling the device to adhere to the stomach (or other GI) walls, thus resisting gastric emptying

• epichlorohydrin cross-linked pectins used as colon specific drug delivery carriers to prolong the residence time.

• omeprazole magnesium as multiple-unit pellet systems (MUPS).

Several difficulties are faced in designing controlled release systems for better absorption and enhanced bioavailability. One of such difficulties is the inability to confine the dosage form in the desired area of the gastrointestinal tract. Drug absorption from the gastrointestinal tract is a complex procedure and is subject to many variables. It is widely acknowledged that the extent of gastrointestinal tract drug absorption is related to contact time with the small intestinal mucosa.

Microencapsulation:

Microencapsulation is a rapidly expanding technology. As a process it is a means of applying relatively thin coatings to small particles of solids or droplets of liquids dispersions. Microencapsulation is arbitrarily differentiated from macro coating techniques in that the former involves the coating of particle ranging from several tenths of a micron to 5000 microns in size.(Herbert A. Lieberman *et al.,* 1987)

Microencapsulation provides the means of converting liquids to solids, of altering colloidal and surface properties, of providing environmental protection and controlling the release characteristics.

Microencapsulation is a process whereby small discrete solid particles or small liquid droplets Aare surrounded or enclosed, by an intact shell. Two major classes of microencapsulation have involved i.e., chemical and physical.

The first class of microencapsulation method involves polymerization during the process of preparing the microcapsules. The second type involves controlled precipitation of a polymeric solution where in physical changes usually occur (Chowdary K.P.R and Sri Ram Murthy 1998).

MICROENCAPSULATION PROCESS

Basic microencapsulation process can be divided into chemical and mechanical.

Chemical process involved

- Complex coacervation
- Polymer-polymer compatibility
- Interfacial polymerization in liquid media

- In situ polymerization
- In liquid drying
- Thermal and ionic gelation in liquid media.

Mechanical process involved

- Spray drying
- Spray coating
- Fluidized bed coating
- Electrostatic deposition
- Centrifugal extrusion
- Spinning disk
- Polymerization at liquid gas or solid gas interface
- Pressure extraction or spraying into solvent extraction bath(Simon Bonita 1983).

IDEAL CHARACTERISTICS OF DRUG FOR MICROENCAPSULATION (D.M Brahmankar *et al* 2002**).**

Particle size requirement:

The lower the molecular weight, faster and complete is the absorption of the drug. The drugs having size 150-600 daltons they can easily diffuse through the membrane but diffusivity is inversely related to molecular size.

The drug or protein should not be adversely affected by the process.

Reproducibility of the release profiles and method.

Stability:

Drugs unstable in GI environment cannot be administered as oral controlled release formulation because of bioavailability problems. E.g. Nitroglycerine.

There should be no toxic product associated with the final product

Therapeutic range:

A candidate drug for controlled drug delivery system should have a therapeutic range wide enough such that variations in the release rate do not result in a concentration beyond this level.

Therapeutic index:

The ratio of maximum safe concentration to the minimum effective concentration of the drug is called as the therapeutic index. The release rate of a drug with narrow therapeutic index should be such that the plasma concentration is attained between the therapeutically safe and effective range. It is necessary because such drugs have toxic concentration nearer to their therapeutic range.

Elimination half life:

Smaller the half life larger the amount of drug to be incorporated in the controlled release dosage form. Drugs with $t_{1/2}$ in the range of 2 to 4 hours makes a good candidates for such a system e.g. Propanolol.

Plasma concentration response relationship:

Drug whose pharmacologic activity is independent of its concentration are poor candidates for controlled release systems.

MICROENCAPSULATION OF PHARMACEUTICALS IS UNDERTAKEN FOR THE FOLLOWING APPLICATIONS:

Microencapsulation has been employed to provide protection to the core material against the atmospheric effects. The separation of incompatible substances, for example pharmaceutical eutectics, has been achieved by encapsulation. Toxic chemicals such as insecticides may be microencapsulated to reduce hazards. Also the hygroscopic properties many core material such as sodium chloride may be reduced by microencapsulation.

Many drugs have been microencapsulated to reduce the gastric and other gastrointestinal tract irritation. The local irritation and release properties of a number of topically applied products can be altered by microencapsulation. This process also used to mask the taste of bitter drugs.

Microencapsulation has been widely employed in the design of controlled release and sustained release dosage forms. It is the most recent addition to oral prolonged release mechanism. The use of microencapsulation for the production of sustained release dosage forms has been widely employed in the last 30 years since the successful introduction by smith, nine and French in the early 1950's.

The physical nature of the core materials and the particle size ranges applicable to each process are given in following table.

The process generally is considered to be applicable only to the encapsulation of solid core materials as indicated in following table:

Microencapsulation process and their applicablities

Microencapsulation Process	**Applicable Core Material**	**Approximate Particle Size In µm**
Air suspension	Solids	35-5000
Coacervation phase separation	Solids and liquids	2-5000
Multi orifice centrifugal	Solids and liquids	1-5000
Pan coating	Solids	600-5000
Solvent evaporation	Solids and liquids	5-5000
Spray drying and congealing	Solids and liquids	600

TWO GENERAL STRUCTURES ARE EXISTS- MICROCAPSULES AND MICROPARTICLES

Microcapsules are a system that contains a well defined core and a well defined envelop. The core can be solid, liquid or gas: the envelope is made of continuous, porous, non porous, polymeric phase. The drug can be dispersed inside the microcapsule as solid particulates with regular or irregular shapes, pure or dissolved solution suspension, emulsion or a combination of suspension and emulsion.

A micro particle is a structure made of a continuous phase of one or more miscible polymers in which particulate drug is dispersed either at macroscopic or molecular levels. However the difference between the two systems is the nature of the micro particle matrix in which no well defined wall or envelop exists (Chowdary *et al.,* 1998).

On the basis of classification these microcapsules and microspheres can be prepared by following techniques

- Single emulsion technique
- Double emulsion technique
- Polymerization technique
- Normal polymerization technique
- Interfacial polymerization technique
- Phase separation polymerization technique
- Spray drying and spray congealing technique
- Solvent extraction technique
- Solvent evaporation technique
- Solvent diffusion technique
- Ionotropic gelation technique

Ionotropic gelation technique

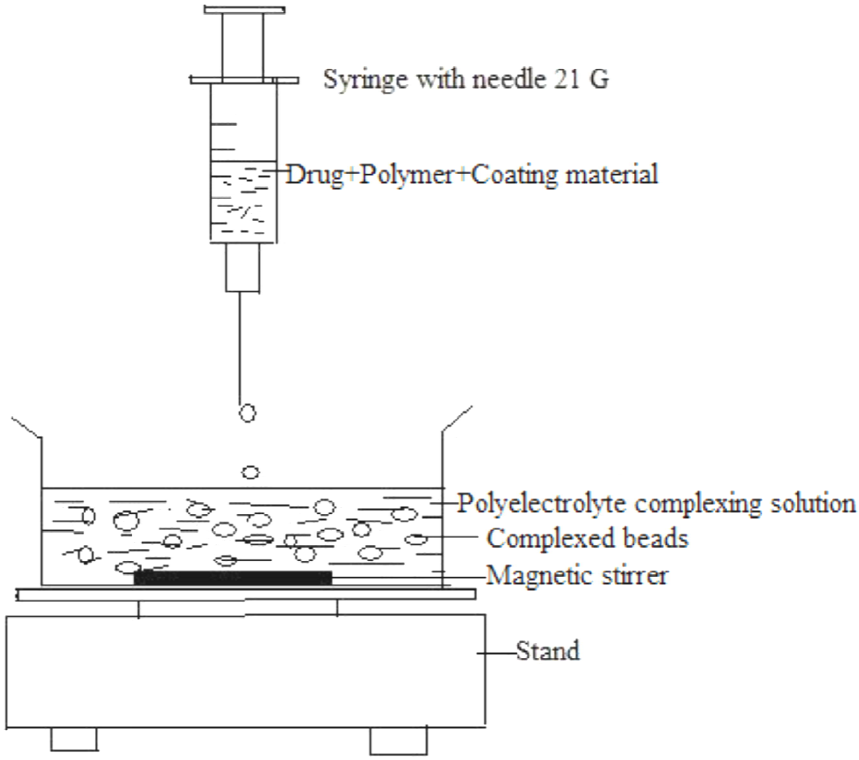


Fig. Schematic diagram of the preparation of beads by ionotropic gelation

Ionotropic gelation is based on the ability of polyelectrolytes to cross link in the presence of counter ions to form beads. Since, the use of alginates, gellan gum, chitosan, and carboxymethyl cellulose for the encapsulation of drug and even cells, ionotropic gelation technique has been widely used for this purpose. The natural polyelectrolytes inspite, having a property of coating on the drug core and acts as release rate retardants contains certain anions on their chemical structure. These anions forms meshwork structure by combining with the polyvalent cations and induce gelation by binding mainly to the anion blocks. The beads are produced by dropping a drug-loaded polymeric solution into the aqueous solution of polyvalent cations. The cations diffuses into the

drug-loaded polymeric drops, forming a three dimensional lattice of ionically crossed linked moiety. Biomolecules can also be loaded into these hydrogel beads under mild conditions to retain their three dimensional structure.

Alginate is linear naturally occurring polysaccharide, consisted of D-mannuronic (M) and L-guluronic (G) acids. The ability of alginate to form gel in the presence of multivalent ions has been applied to prepare beads by ionotropic gelation method where the dispersion of alginate and material to be encapsulated is added dropwise into multivalent ion solution. The contact of droplets with multivalent ions results in instantaneous formation of gel spheres containing uniformly dispersed material throughout the crosslinked alginate matrix. The size of wet beads is dependent on size of a droplet of polymer dispersion, which is influenced by diameter of a nozzle and viscosity of polymer dispersion. However, the drying may influence the size and shape of dry beads.

Sodium alginate (algin) is the purified carbohydrate product isolated from brown seaweads. Algin consists chiefly of the sodium salt of alginic acid, which is a linear copolymer of 1,4- linked mannopyranosyluronic acid and 1,4- linked gulopyranosyluronic acid units as shown in fig

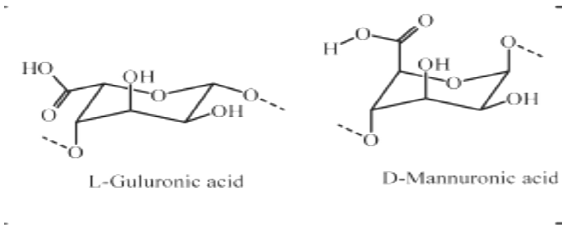


Fig: Structure of alginic acid

Alginate has been investigated as a carrier material in different controlled release systems. It was employed in the preparation of controlled release microspheres or minimatrices for a variety of medicinal agents including protein drugs (George and Abraham, 2006; Raj and Sharma, 2003), metoclopramide and cisapride (Al-Musa *et al.,* 1999), diclofenac (Fernandez-Hervas *et al.,* 1998), indomethacin, propranolol (Lim and Wan, 1997), and gentamicin. Furthermore, alginic acid was used to encapsulate chitosan bioadhesive microspheres, and *vice versa,* for intestinal drug delivery (Gaserod *et al.,* 1998).

Algin is characterized with useful gel-forming properties when mixed with different polyvalent cations (Aslani and Kennedy, 1996). In particular, algin forms stable complexes with calcium ions that seem to assume the “egg box” model (Li *et al.,* 2007). Calcium alginate has found applications in a number of gelation purposes including the formation of a firm gel for the preparation dental impressions and in the preparation of matrices for drug delivery (Aslani and Kennedy, 1996;). The ratio of mannuronic acid to guluronic acid strongly influences the drug releasing properties of calcium alginate beads.

CHAPTER II

MUCOADHESIVE DRUG DELIVERY SYSTEM: A REVIEW

Bioadhesives are natural polymeric materials that act as adhesives. The term is sometimes used more loosely to describe glue formed synthetically from biological monomers such as sugars, or to mean a synthetic material designed to adhere to biological tissue. The term bioadhesion refers to any bond formed between two biological surfaces or a bond between biological and synthetic surfaces. (J.H. Bhatt, 2009). It may be defined as attachment of synthetic biological macromolecules to a biological tissue. A more specific term than bioadhesion is mucoadhesion.

Mucoadhesion is the relatively new and emerging concept in drug delivery. Mucoadhesion is the special case of bioadhesion where the biological tissue is an epithelium covered by mucus. (Sumit Anand Abnawe, 2009). Most mucosal surfaces such as in the gut or nose are covered by a layer of mucus.

Adhesion of a matter to this layer is hence called mucoadhesion. Mucoadhesion keeps the delivery system adhering to the mucus membrane. Mucoadhesion can be defined as the ability of synthetic or biological macromolecules to adhere to mucosal tissues. The concept of mucoadhesion is one that has the potential to improve the highly variable residence times experienced by drugs and dosage forms at various sites in the gastrointestinal tract, and consequently, to reduce variability and improve efficacy.

These systems remain in close contact with the absorption tissue, the mucous membrane, releasing the drug at the site of action leading to an increase in bioavailability. (Flavia Chiva Carvalho *et al.,* 2010).

Mucoadhesive drug delivery system prolong the residence time of the dosage form at the site of application or absorption and facilitate an intimate contact of the dosage form with the underline absorption surface and thus contribute to improved and / or better therapeutic performance of the drug (G.S.Asane, 2007). The mucoadhesive drug delivery system may include the following

1. Buccal delivery system.
2. Sublingual Delivery system.
3. Vaginal delivery system.
4. Rectal delivery system.
5. Nasal delivery system.
6. Ocular delivery system.
7. Gastro intestinal delivery system. (G.S.Asane, 2007; S.B.Patil et al., 2006, G.C. Rajput, 2010)

Their ability to stick to mucous membranes attracted attention as a pathway for resolving the problem of low bioavailability of traditional delivery systems used in the oral cavity and on the surface of the eye or other organs where movement of tissues or production of various secretions prevents prolonged retention of the medicinal agent. The reasons that the oral route achieved such popularity may be in part attributed to its ease of administration as well as the traditional belief that by oral administration the drug is well absorbed as the food stuffs that are ingested daily. (G.S.Asane, 2007).

In the exploration of oral controlled release drug administration, one encounters three areas of potential challenge.

1. Development of a drug delivery system: To develop a viable oral controlled release drug delivery system capable of delivering a drug at a therapeutically effective rate to a desirable site for duration required for optimal treatment.

2. Modulation of gastro intestinal transit time: To modulate the GI transit time so that the drug delivery system developed can be transported to a target site or to the vicinity of an absorption site and reside there for prolonged period of time to maximize the delivery of a drug dose.

3. Minimization of hepatic first pass elimination: If the drug to be delivered is subjected to extensive hepatic first pass elimination, preventive measures should be devised to either bypass or minimize the extent of hepatic metabolic effect.

MUCOADHESIVE DRUG DELIVERY SYSTEM

DEFINITION

Adhesion can be defined as the bond produced by contact between a pressure - sensitive adhesive and a surface (Jimenez-Castellanous, 1993). The American Society of testing and materials has defined it as the state in which two surfaces are held together by interfacial forces, which may consist of valence forces, interlocking action or both. When the adhesion involves mucus or mucus membrane it is termed as mucoadhesion (J.H.Bhatt, 2009)

CONCEPTS

In biological systems, four types of bioadhesion can be distinguished as follows:-

1. Adhesion of a normal cell on another normal cell.

2. Adhesion of a cell with a foreign substance.

3. Adhesion of a normal cell to a pathological cell.

4. .Adhesion of an adhesive to a biological substance.

MUCOUS MEMBRANE

Mucous membranes are the moist linings of the orifices and internal parts of the body that are in continuity with the external surface. They cover, protect, and provide secretory and absorptive functions in the channels and extended pockets of the outside world that are incorporated in the body. Mucus is a translucent and viscid secretion, which forms a thin, continuous gel blanket adherent to mucosal epithelial surface. The mean thickness of this layer varies from about 50-450 μm in humans. It is secreted by the goblet cells lining the epithelia or by special exocrine glands with mucus cells acini. The exact composition of the mucus layer varies substantially, depending on the species, the anatomical location and pathological states. (G.C. Rajput et.al., 2010). They secrete a viscous fluid known as mucus, which acts as a protective barrier and also lubricates the mucosal membrane. Mucosal membranes of human organism are relatively permeable and allow fast drug absorption They are characterized by an epithelial layer whose surface is covered by mucus (Flavia Chiva Carvalho et.al.,2010) The primary constituent of mucus is a glycoprotein known as mucin as well as water and inorganic salts.(S.Ganga,2007). However, it has general composition.

Table 1: Composition of Mucous Membrane

EXAMPLES OF MUCOSA

· Buccal mucosa.

· Oesophageal mucosa.

· Gastric mucosa.

· Intestinal mucosa.

· Nasal mucosa.

· Olfactory mucosa.

· Oral mucosa.

· Bronchial mucosa.

· Uterine mucosa.

· Endometrium (mucosa of the uterus).

· Penile mucosa.

FUNCTIONS OF MUCOUS LAYER

The mucous layer, which covers the epithelial surface, has various roles (G.C. Rajput *et al.,* 2010).

1. Protective role. 2. Barrier role. 3. Adhesion role. 4. Lubrication role 5.Mucoadhesion role.

1. **PROTECTIVE ROLE:** The Protective role results particularly from its hydrophobicity and protecting the mucosa from the lumen diffusion of hydrochloric acid from the lumen to the epithelial surface. (G.C.Rajput *et al.,* 2010)

2**. BARRIER ROLE:** The role of mucus layer as barrier in tissue absorption of drugs and other substances is well known as its influence the bioavailability of the drugs. The mucus constitutes diffusion barrier for molecules, and especially against drug absorption diffusion through mucus layer depends on molecule charge, hydration radius, ability to form hydrogen bonds and molecular weight.

3. **ADHESION ROLE:** Mucus has strong cohesive properties and firmly binds the epithelial cells surface as a continuous gel layer (G.C.Rajput *et al.,* 2010).

4. **LUBRICATION ROLE:** An important role of the mucus layer is to keep the membrane moist. Continuous secretion of mucus from the goblet cells is necessary to

compensate for the removal of the mucus layer due to digestion, bacterial degradation and solubilisation of mucin molecules. (G.C.Rajput *et al.,* 2010).

5. **MUCOADHESION ROLE:** One of the most important factors for bioadhesion is tissue surface roughness. (G.S.Asane, 2007), Adhesive joints may fail at relatively low applied stresses if cracks, air bubbles, voids, inclusions or other surface defects are present. Viscosity and wetting power are the most important factors for satisfactory bioadhesion (G.C.Rajput *et al.,* 2010).

At physiological pH, the mucus network may carry a significant negative charge because of the presence of sialic acid and sulphate residues and this high charge density due to negative charge contributes significantly to the bioadhesion. (G.C.Rajput *et al.,* 2010)

NEED OF MUCOADHESIVE:

· Controlled release.

· Target &localised drug delivery.

· By pass first pass metabolism.

· Avoidance of drug degradation.

· Prolonged effect.

· High drug flux through the absorbing tissue.

· Reduction in fluctuation of steady state plasma level. (Sumit Anand Abnawe, 2009)

An ideal dosage form is one, which attains the desired therapeutic concentration of drug in plasma and maintains constant for entire duration of treatment. This is possible through administration of a conventional dosage form in a particular dose and at particular frequency. In most cases, the dosing intervals much shorter than the

half life of the drug resulting in a number of limitations associated with such a conventional dosage form are as follows:

· Poor patient compliance; increased chances of missing the dose of a drug with short half-life for which frequent administration is necessary.

· A typical peak plasma concentration time profile is obtained which makes attainment of steady state condition difficult.

· The unavoidable fluctuation in the drug concentration may lead to under medication or over medication as the steady state concentration values fall or rise beyond in the therapeutic range.

· The fluctuating drug levels may lead to precipitation of adverse effects especially of a drug with small therapeutic index whenever overmedication occurs (M. Bramhankar and S.B. Jaiswal, 1995).

ADVANTAGES OF MUCOADHESIVES

· A prolonged residence time at the site of drug action or absorption.

· A localization of drug action of the delivery system at a given target site.

· An increase in the drug concentration gradient due to the intense contact of particles with the mucosal. (G.C.Rajput *et al.,* 2010).

· A direct contact with intestinal cells that is the first step before particle absorption.

· Ease of administration.

· Termination of therapy is easy.

· Permits localization of drug to the oral cavity for a prolonged period of time.

· Can be administered to unconscious patients.

· Offers an excellent route, for the systemic delivery of drugs with high first pass metabolism, thereby offering a greater bioavailability.

· A significant reduction in dose can be achieved there by reducing dose related side effects.

· Drugs which are unstable in the acidic environment are destroyed by enzymatic or alkaline environment of intestine can be administered by this route. Eg. Buccal, sublingual and vaginal.

· Drugs which show poor bioavailability via the oral route can be administered conveniently.

· It offers a passive system of drug absorption and does not require any activation.

· The presence of saliva ensures relatively large amount of water for drug dissolution unlike in case of rectal and transdermal routes.

· Systemic absorption is rapid (G.C.Rajput *et al.,* 2010).

· This route provides an alternative for the administration of various hormones, narcotic analgesic, steroids, enzymes, cardiovascular agents etc.

· The buccal mucosa is highly perfused with blood vessels and offers a greater permeability than the skin.

· Less dosing frequency.

· Shorter treatment period.

· Increased safety margin of high potency drugs due to better control of plasma levels.

· Maximum utilization of drug enabling reduction in total amount of drug administered.

· Improved patient convenience and compliance due to less frequent drug administration.

· Reduction in fluctuation in steady state levels and therefore better control of disease condition and reduced intensity of local or systemic side effects. (G.C.Rajput *et al.,*2010).

Despite the several advantages associated with oral controlled drug delivery systems, there are so many disadvantages, which are as follows:

· Basic assumption is drug should absorbed throughout GI tract

· Limited gastric residence time which ranges from few minutes to 12 hours which lead to unpredictable bioavailability and time to achieve maximum plasma level. (G.C.Rajput *et al.,* 2010).

LIMITATIONS

· Drug administration via the buccal mucosa has certain limitations

· Drugs, which irritate the oral mucosa, have a bitter or unpleasant taste, odour, cannot be administered by this route.

· Drugs, which are unstable at buccal pH cannot be administered by this route.

· Only drugs with small dose requirements can be administered.

· Drugs may swallow with saliva and loses the advantages of buccal route.

· Only those drugs, which are absorbed by passive diffusion, can be administered by this route.

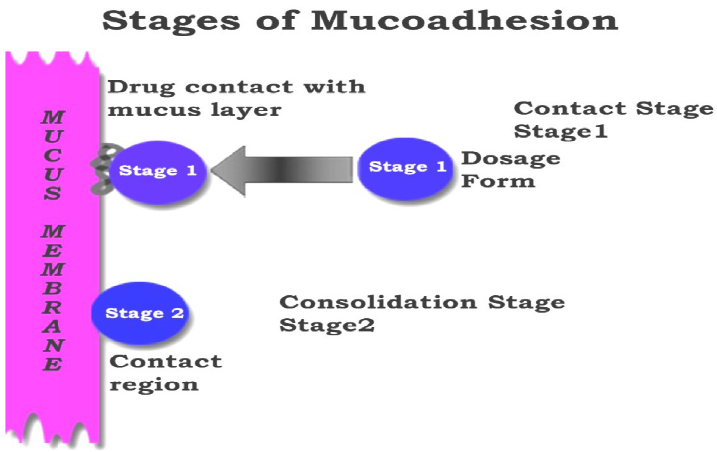
· Eating and drinking may become restricted.

· Swallowing of the formulation by the patient may be possible.

· Over hydration may lead to the formation of slippery surface and structural integrity of the formulation may get disrupted by the swelling and hydration of the bioadhesive polymers.

STAGES OF MUCOADHESION

1. CONTACT STAGE 2. CONSOLIDATION STAGE.



MECHANISM OF MUCOADHESION

The concept of mucoadhesion is one that has the potential to improve the highly variable residence times experienced by drugs and dosage forms at various sites in the gastrointestinal tract, and consequently, to reduce variability and improve efficacy. Intimate contact with the mucosa should enhance absorption.

The mechanisms responsible in the formation of bioadhesive bonds are not fully known, however most research has described bioadhesive bond formation as a three step process:-

STEP1: Wetting and swelling of polymer

STEP2: Interpenetration between the polymer chains and the mucosal membrane.

STEP3: Formation of Chemical bonds between the entangled chains**. (**John D. Smart, 2005**)**

Step 1:-The wetting and swelling step occurs when the polymer spreads over the surface of the biological substrate or mucosal membrane in order to develop an intimate contact with the substrate. (J.H.Bhatt, 2009; Helene Hagerstrom, 2003) This can be readily achieved for example by placing a bioadhesive formulation such as a

tablet or paste within the oral cavity or vagina. Bioadhesives are able to adhere to or bond with biological tissues by the help of the surface tension and forces that exist at the site of adsorption or contact. Swelling of polymers occurs because the components within the polymers have an affinity for water.

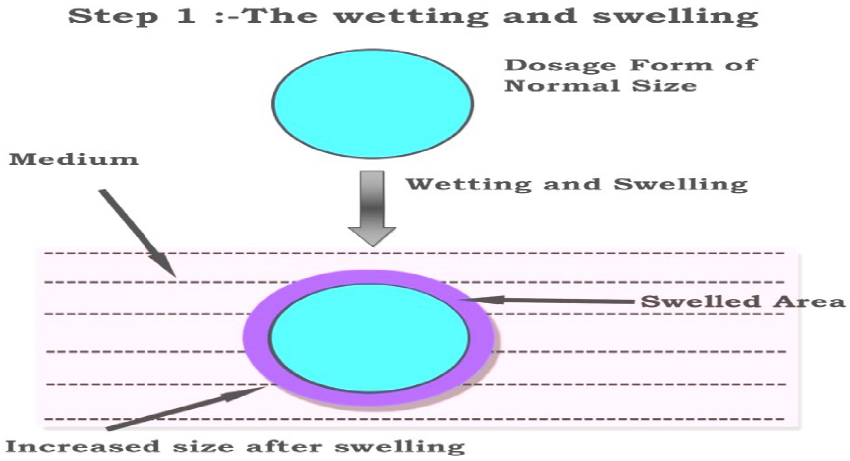
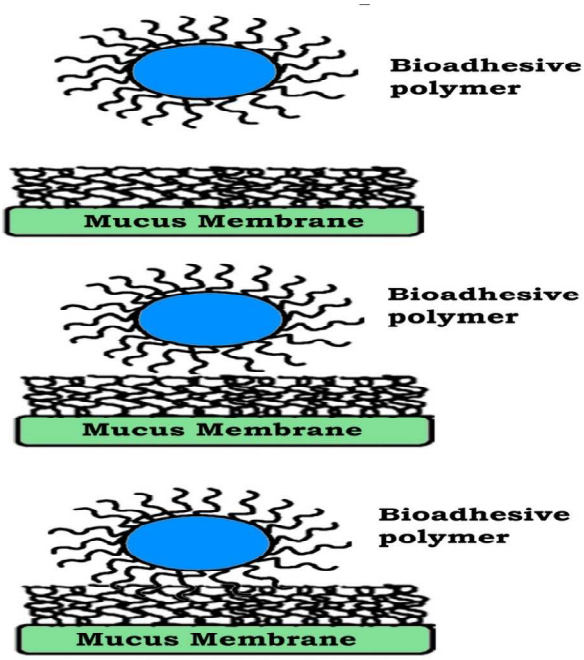


Figure: Wetting and Swelling of Polymer

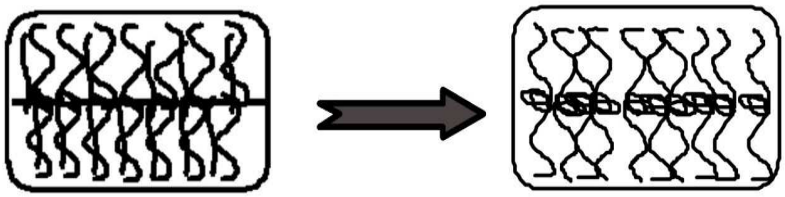
Step 2: The surface of mucosal membranes are composed of high molecular weight polymers known as glycoproteins. In this step interdiffusion and interpenetration take place between the chains of mucoadhesive polymers and the mucous gel network creating a great area of contact.(Helene Hagerstrom, 2003; Hemanta Kumar Sharma et.al., 2009) The strength of these bond depends on the degree of penetration between the two polymer groups. In order to form strong adhesive bonds, one polymer group must be soluble in the other and both polymer types must be of similar chemical structure. (Sheila Aidoo, 2009, John D. Smart, 2005).

Step 3:- In this step entanglement and formation of weak chemical bonds as well as secondary bonds between the polymer chains mucin molecule (Helene Hagerstrom, 2003). The types of bonding formed between the chains include primary bonds such as covalent bonds and weaker secondary interactions such as van der Waals

Interactions and hydrogen bonds. Both primary and secondary bonds are exploited in the manufacture of bioadhesive formulations in which strong adhesions between polymers are formed. (Helene Hagerstrom, 2003).



Formation of Chemical Bond



Interpenetration between polymer and mucus

Entanglement of polymer and mucus

Prabhakar reddy veera reddy *et al.,* 2011 Prepared and evaluated mucoadhesive cefdinir microcapsules. The mucoadhesive microcapsules were prepared by using various concentrations of three different polymers, namely, chitosan, carbopol 934p and methyl cellulose as wall materials and cefdinir as the core material employing orifice ionic gelation method. The microcapsules were found to be spherical with particle size ranging from 765±20 to 985±10µm and encapsulation efficiencies in the range of 55% - 92%. The formulation containing carbopol 934p as mucoadhesive polymer was found to be best with particle size 946±10µm. the ex vivo wash off test showed that the mucoadhesion after 1 hr was extended for more than 12hr. FT-IR spectra indicate that there was no interaction between drug and the polymers used in the formulation. Cefdinir is better absorbed from the upper part of GIT; it suffers from low oral bioavailability (20-30%), shorter biological half life (1-2h) and less transit time. Thus it can be concluded that microcapsules prepared using carbopol 934p have promising properties for use as mucoadhesive carrier to increase residence time of cefdinir.

Harshad Parmar *et al.,* 2011 Formulated, optimized and *in vitro* characterization of mucoadhesive microparticle. Mucoadhesive microparticle of Itopride HCl was design in order to obtain a unique drug delivery system which would remain in the stomach and prolong the residence time at the absorption site by intimate contact with the mucus layer thereby increase bioavailability, reduce the frequency of dose administration and also to prolong the drug release. The mucoadhesive microparticles were prepared by Orifice ionic gelation method using sodium alginate in combination with carbopol 934 and HPMC K15. Entrapment efficiency was in the range of 41.32 to 81.68 %. Microparticle exhibited good mucoadhesive property in the *in vitro* wash off test and revealed that Carbopol 934 had greater mucoadhesive strength than that of HPMC K15. Itopride HCl release from this

mucoadhesive microparticle was slow and showed sustained release. SEM study revealed that microparticles were discrete, spherical and free flowing. Stability study of optimized batch was carried out and drug content found was retained with permissible limits and there was no significant difference in the drug content.

Ashok kumar. A *et al.,* 2011 formulated and evaluated mucoadhesive microcapsules of metformin hcl with gum karaya. The objective of this work was to develop mucoadhesive microcapsules of Metformin Hcl for controlled release. Metformin Hcl microcapsules were prepared with a coat consisting of alginate and Gum Karaya by employing Ionotropic Gelation process and Emulsification Ionotropic Gelation process. The microcapsules were evaluated for flow properties, Carr's index, hausner ratio, micro-encapsulation efficiency, drug release characteristics, surface characteristics; compatibility studies and mucoadhesive properties. As hausner ratio was less than 1.25 and Carr's index values were less than 25 from both the methods, hence they were found to be free flowing. Sharp endothermic peaks were noticed from the microcapsules formulated with two different techniques at 226ºC indicating the compatibility between the drug and the polymer Gum Karaya. Metformin Hcl release from the microcapsules was slow and followed zero order kinetics ($r > 0.98$) and followed non–fickian (n value 0.5 to 1) release and depended on the coat: core ratio and the method employed in the preparation of microcapsules. Among the two methods Emulsification Ionotropic Gelation method was found to be more suitable for Controlled release of Metformin Hcl over a long period of time. These microcapsules were subjected to in-vitro wash-off test and exhibited good mucoadhesive property.

Sivakumar R *et al.,* 2011 designed mucoadhesive hydropilic beads entrapped with ketoprofen for delivery into small intestine. The purpose of this study was to develop and

evaluate pH dependent multiparticles of ketoprofen loaded mucoadhesive beads to target the small intestine. The hydrogel beads were prepared by inotropic gelation method using sodium alginate, pectin and xanthan gum as polymers. The prepared gel beads were coated with 1 % chitosan. The obtained beads were filled into hard gelatin capsules and enteric coated with Eudragit L100. The beads were evaluated for particle size, morphology, encapsulation efficiency, *in vitro* release, and mucoadhesion. The size of microbeads ranged from 1mm to 2mm and the encapsulation of ketoprofen beads was between 60 to 70%. The release of ketoprofen from the gel beads at pH 6.8 was initially fast followed by a slower and more controlled release. The drug release from the beads was found to follow case II transport mechanism (n>0.85) and was independent of time, which corresponds with zero-order kinetics.

Mohammed G Ahamed *et al.*, 2010 Formulated and evaluated gastric mucoadhesive drug delivery systems of captopril. Gastro-retentive beads of captopril were prepared by orifice ionic gelation method in 1:1 and 9:1 ratio of alginate along with mucoadhesive polymers viz;hydroxy propyl methyl cellulose, carbopol 934P, chitosan and cellulose acetate phthalate. It was observed that as the alginate proportion was increased, the average size of beads also increased. Photomicrographs revealed that the beads were spherical in shape. Alginate chitosan (9:1) beads showed excellent microencapsulation efficiency (89.7 percent). Alginate-Carbopol 934P exhibited maximum efficiency of mucoadhesion in 0.1 N hydrochloric acid (44 percent for 1:1 and 22 percent for 9:1) at the end of 8 hours, whereas least mucoadhesion was observed with alginate-Cellulose acetate phthalate beads. The *in vitro* release studies were carried out in 0.1 N hydrochloric acid and the release were found to be more sustained with Alginate-chitosan beads (9:1) than Alginate-Carbopol 934P (1:1)

beads. The alginate-cellulose acetate phthalate beads showed the better sustained release as compared to all other alginate polymer combinations.

Raghavendra V. Kulkarni *et al.,* 2010 Reported carboxymethyl cellulose – aluminium hydrogel microbeads for prolonged release of simvastatin. Carboxy methyl cellulose based hydrogel microbeads loaded with simvastatin were prepared using ionotrophic gelation method.the beads were characterized by differential scanning calorimetric analysis, and scanning electron microscopy. DSC studies confirmed the amorphous dispersion of the drug in the hydrogel matrix. The effect of cross linking agent and polymer concentration on drug release was studied. Increase in concentration of cross linking agent and polymers decreased the release rate of simvastatin. The release data were fitted to an empirical equation to determine the transport mechanism. Drug release followed anomalous/non- fickian transport mechanism.

Bhanja S.B. *et al.,* 2010 Prepared and evaluated of mucoadhesive microcapsules of acyclovir. Acyclovir microcapsules with a coat consisting of alginate and a mucoadhesive polymer such as carbopol 934P and hydroxypropyl methyl cellulose E 15 V were prepared by an ionotropic gelation technique, where gelation was achieved with oppositely charged counter ions to form microcapsules. The microcapsule prepared were found to be spherical to near spherical and without aggregation discrete and free flowing. The percent yield, drug entrapment and drug content in all formulations were good. The microencapsulation efficiency of all the formulations was in the range of 38.60 to 70.35%. The average particle size was found to be in the range of 409.25 to 725µm. All the formulations show excellent flowability as expressed in term of angle of repose (<25) and the formulation FC1 show good flowability. A percentage of moisture loss was calculated for all the prepared acyclovir

microcapsules and was found to be within limit. The swelling indexes of microcapsules were found satisfactory. All the formulations were found to release Acyclovir in a controlled manner for a prolonged period over 8 hour. All formulations were followed first order kinetics and formulations have diffusion controlled release pattern. The mucoadhesion of the selected microcapsules were studied by *in vitro wash off* test according to their *in vitro* drug release profile. The result of the *in vitro wash off* test fairly showed good mucoadhesive property of the microcapsules prepared from sodium alginate. The percentage of moisture loss was found in a range 2.24 to 8.81%.

Sangeetha. S *et al.,* 2010 Designed gastroretentive beads of theophylline by ionotrophic gelation. A Gastroretentive bead of theophylline by ionotropic gelation was formulated in two different combinations such as sodium alginate along with guar gum and sodium alginate with hydroxy ethyl cellulose. The gas forming agent's calcium carbonate was also added in four different concentrations. The formulated beads were then evaluated for particle size, drug content, floating properties and *invitro* dissolution. The *invitro* release study showed about 98-99% of drug release at the end of 8 hrs with good buoyancy effect for the batch formulated with the combination of sodium alginate and guar gum. The *invitro* release mechanism was found to be anomalous diffusion with first order kinetics.

Chowdary P.K. *et al.,* 2010 Designed, developed and evaluated frusemide loaded micropellets prepared by ionotropic gelation method. Frusemide is a representative of loop diuretics, which is commonly indicated for acute or chronic renal failure. In low dose it is also used for the treatment of chronic hypertension. It has got pH independent solubility behavior. The half life of Frusemide is 1.5 hr and it is predominantly metabolized in kidney. The micro beads were prepared by the ionotropic gelation of sodium alginate in calcium

chloride solution, which were further made sustained by using different acrylic polymers namely Eudragit NE30D, Eudragit S100. The prepared micro beads were evaluated mainly for the sustain release of the drug and the effect of these polymers on the release profile of the drug has been reported in this study. Different formulations were prepared using Eudragit NE30D (F1, F2); and Eudragit S100 (F3, F4) at concentration 2%, 4%w/w. The final formulations were subjected to several characterization studies like, general appearance, particle size determination, rheological studies, Scanning Electron Microscopy, moisture content, loose surface crystals study, drug content and % drug encapsulation efficiency and *in vitro* drug release study. The method had resulted in good encapsulation efficiency and micron sized alginate spheres. The drug release was found to be sustained as only 72 % to 90 % of the cumulative drug release were observed in all formulations after 9 hours, which found to follow the Higuchi's diffusion model. Among all formulations, the formulation F2 with Eudragit NE30D 4%w/w showed high encapsulation efficiencies and maximum prolongation of drug release.

Hitesh patel *et al.,* 2010 Reported ionotrophic gelation technique used for microencapsulation of antihypertensive drug. Micropellets of verapamil hydrochloride were formulated by ionotropic gelation technique using sodium alginate, hydroxy propyl methyl cellulose and hydroxy propyl cellulose. Prepared micropellets were evaluated for flow behaviour, drug entrapment efficiency, *in-vitro* dissolution and stability studies, including scanning electron microscopy and optical microscopy. Of the nine formulations prepared and evaluated formulations F3, F6 and F9 were found to show satisfactory results. The release of the drug from the micropellets was found to be following Non-Fickian diffusion, Drug diffusion coefficient and correlation coefficient were also assessed using various

mathematical models. From the study it was concluded that, prolonged release Verapamil hydrochloride micropellets can be achieved with success using ionotropic gelation technique.

Maya Davidovich-Pinhas and Havazelet Bianco-Peled 2010 reported quantitative analysis of alginate swelling. The swelling behavior of physically cross-linked polysaccharides is not fully understood despite its significance in many applications such as drug delivery. In this study the swelling behavior of three types of alginate were characterized experimentally at various calcium concentrations. Additionally, equilibrium swelling data was analyzed in terms of Flory and rubber elasticity theories, which were developed for chemically cross-linked networks. This analysis suggested that these theories are not applicable for alginate. In particular, an increase in the number of monomeric units between cross-links was observed at a higher calcium concentration, whereas the theory predicts the opposite. The kinetics of the swelling process was also analyzed experimentally and theoretically. The experimental data was found to obey second-order kinetics. Moreover, a decrease in the swelling rate constant with elevated calcium concentration was observed. Lastly, it is indicated that the unusual swelling behavior of alginate could be attributed to a lateral chain association.

Jayvadan patel *et al.,* 2010 Formulated and evaluated propranolol hydrochloride-loaded carbopol-934P/ethyl cellulose mucoadhesive microspheres. Propranolol hydrochloride mucoadhesive microspheres, containing carbopol-934P as mucoadhesive polymer and ethyl cellulose as carrier polymer, were prepared by an emulsion solvent evaporation technique. Results of preliminary trials indicated that the quantity of emulsifying agent, time for stirring, drug-to-polymers ratio, and speed of rotation affected various characteristics of microspheres. Microspheres were discrete, spherical, free-flowing and showed a good percentage of drug entrapment efficiency. An *in-vitro* mucoadhesive test showed that propranolol hydrochloride

mucoadhesive microspheres adhered more strongly to the gastric mucous layer and could be retained in the gastrointestinal tract for an extended period of time. The best batch exhibited a high drug entrapment efficiency of 54 %; 82% mucoadhesion after 1 h and particle size of 110 μm. A sustained pattern of drug release was obtained for more than 12 h. The drug-to-polymer-to-polymer ratio had a more significant effect on the dependent variables. The morphological characteristics of the mucoadhesive microspheres were studied under a scanning electron microscope. The results showed a sustained anti-hypertensive effect over a longer period of time in case of mucoadhesive microspheres, compared to the powder. In conclusion, the prolonged gastrointestinal residence time and slow release of propranolol hydrochloride resulting from the mucoadhesive microspheres, could contribute to the provision of a sustained anti-hypertensive effect.

Ravindra Reddy K and Sabitha Reddy P 2010 studied effect of different Co-polymers on Sodium Alginate Microcapsules Containing Isoniazid. The present investigation was designed to develop, characterize and evaluate mucoadhesive microcapsules of isoniazid employing various mucoadhesive polymers for prolonged gastric intestinal absorption. Sodium alginate is an anionic polymer which can be easily cross-linked with calcium chloride. This is because the calcium ions are bound to carboxylate residues of both mannuronic acid and glucouronic acid which are components of sodium alginate. The complexation between calcium ions and sodium alginate leads to controlled release of drugs. Three different formulations were prepared with core: coat ratio 1:2 and by using three different co-polymers in the ratio of 5:1(polymer: co-polymer) by employing orifice ionic gelation method. The method produced discrete, free flowing and spherical microcapsules ratios. The prepared microcapsules were evaluated for SEM analysis, sieve analysis, drug content, encapsulation efficiency, swelling studies and compared with pure drug. The

microcapsules obtained were spherical, discrete and free flowing. The release depended on the type of copolymer and size of microcapsules. *In-vitro* release studies were carried out in pH 7.4 and 20.83%, 32.40% and 51.54% of the drug was released from F1 (sodium alginate+methyl cellulose), F2 (sodium alginate+Hydroxy propyl methyl cellulose) and F3 (sodium alginate+sodium carboxy methyl cellulose) respectively upto 12 hrs. Drug release was found to be diffusion controlled and followed first order kinetics. The prepared microcapsules showed sustained release over a period of 12 hrs.

Kundlik M. Girhepunje *et al.,* 2010 developed celecoxib loaded microbeads: A Targeted drug delivery for colorectal cancer. Celecoxib is a nonsteroidal anti-inflammatory drug that exhibits anti-inflammatory, analgesic, and antipyretic activities. Recently, considerable interest has been focused on the use of biodegradable polymers for specialized applications such as targeted release of drug formulations; meanwhile, microbeads drug delivery systems using various kinds of biodegradable polymers have been studied extensively during the past two decades. In the present investigation, it was aimed to prepare microbead formulations of celecoxib inclusion complex using sodium alginate and eudragit FS 30-D as a carrier for colonic administration to extend the retention of the drug in order to treat colorectal cancer. Microbead formulations were evaluated for entrapment efficiency, FT-IR, DSC, SEM, *In vitro* drug release, *In vitro* cell line study, Cytotoxicity Screening. Formulation F5 showed 91.99 ± 1.45% entrapment, which was uniformly dispersed and having smooth surface texture in formulation, F5 shown 92.11±2.32% drug release up to 8 hr. Coated Celecoxib microbeads (1:1ratio) showed cytotoxicity against HT-29 cells. DNA Fragmentation study confirms the better anti cancer activity of celecoxib microbeads against human colorectal adenocarcinoma cell line HT-29. Hence the formulations can be effectively tested for its anticancer activity.

Tavakol M *et al.,* 2009 studied sulfasalazine release from alginate-N, O-carboxy methyl chitosan gel beads coated by chitosan. **S**pherical beads were prepared from a water soluble chitosan and alginate with ionic gelation method. Then swollen beads were coated with chitosan. The effect of coating, as well as drying procedure on the swelling behavior of unloaded beads and SA release of drug loaded ones were evaluated in simulated gastrointestinal tract fluid. The rate of swelling and drug release were decreased for air dried and coated beads in comparison with freeze dried and uncoated ones, respectively. No burst release of drug was observed from whole tested beads. chitosan coated beads released approximately 40% of encapsulated drug in simulated and gastric fluid.

Hemanth kumar Sharma *et al.,* 2009 Prepared and evaluated mucoadhesive microbeads containing timolol maleate using mucoadhesive substances of *dillenia indica l*. The microbeads prepared by ionotrophic gelation method and investigated the shape and size of the various microbeads by microscopic studies. To study the effect of drug-mucoadhesive polymer ratio, type and concentration of cross linking agent ($cacl_2$, $bacl_2$, $Al_{2(}so4)_{3,}$) stirring speed and curing time on drug entrapment, swelling index, mucoadhesiveness and in vitro profile in phosphate buffer ph 6.8 for pre-gastric absorption. Drug polymer interaction and surface morphologies are investigated by dsc and SEM studies respectively. The *in vitro* dissolution study were analyzed with various kinetic equations like zero order model, first order model, higuchi model and korsmeyer-peppas model in order to understand the mechanism and kinetics of drug release.

Veena Belgamwar *et al.,* 2009 Formulated and evaluated oral mucoadhesive multiparticulate system containing metoprolol tartarate: An *In vitro-Ex vivo* characterization. It was aimed to prepare mucoadhesive multiparticulate system for oral drug delivery using ionic gelation

technique. Microspheres composed of various mucoadhesive polymers including HPMC of various grades like K4M, K15M, K100M, E50LV, Carbopol of grades 971P, 974P and polycarbophil were prepared. In this technique cross linking of sodium alginate with calcium chloride was done which retarded the release of drug from the mucoadhesive polymer. In the present work Metoprolol tararate was used as a model drug. Interaction studies performed using FTIR spectroscopy revealed that there was no drug to polymer interactions. The preliminary mucoadhesive strength studies performed for various polymers using rotating cylindrical method showed that HPMC had greater mucoadhesive properties than carbopol and polycarbophil. Microspheres so prepared were discrete, bulky, free flowing and showed an average encapsulation efficiency ranging from 50-60%. Particle size of the microspheres, as determined by the optical microscopy was found to be between 400-650µm. The prepared formulations also exhibited a good mucoadhesive strength which was determined in *in vitro* conditions through falling film technique and were compared with *ex vivo* studies. The microspheres so prepared also exhibited a good swelling index which confirmed the strong mucoadhesive property of the formulation. Metoprolol release from the multiparticulate system was regulated and extended until 12 hours and exhibited a non fickian drug release kinetics approaching to zero order, as evident from the release rate exponent values which varied between 0.57 and 0.73. The stability studies performed on the optimized batches at 40°C/75% RH for 90 days indicated no significant change in the physicochemical properties.

Adhiyaman Rajendran and Sanat Kumar Basu 2009 studied alginate-Chitosan particulate system for sustained release of nimodipine. Nimodipine-loaded alginate-chitosan beads were prepared by ionic gelation method using various combinations of chitosan and Ca^{2+} as cations and alginate as anion. The swelling ability and in vitro drug release characteristics of the beads were studied at pH 1.2 and 6.8. Infra-red (IR) spectrometry, scanning electron

microscopy (SEM), differential scanning calorimetry (DSC), x-ray diffraction (XRD), and atomic absorption spectroscopy (AAS) were also applied to investigate the physicochemical characteristics of the drug in bead formulations. The surface morphology, size, and drug loading of the beads varied with increase in the concentration of chitosan and calcium chloride in the gelation medium. The swelling ability of the beads in different pH media was dependent on the presence of a polyelectrolyte complex in the beads and the pH of the media. Both calcium alginate beads and the beads treated with chitosan failed to release the drug at pH 1.2 over the period of study. On the other hand, at pH 6.8, calcium alginate beads released approx. 96 % of drug in 6 hrs, but treatment of the beads with chitosan lowered drug release to 73 %. Drug release mechanism was either "anomalous transport" or "case-II transport". Data from characterization studies indicate that there was no significant change in the physical state of the drug in the bead formulations.

Pandey manisha *et al.*, 2009 Reported controlled release theophylline loaded buoyant sodium alginate microbeads for prolonged release to gastric mucosa. This investigation describes the preparation and *in vitro* evaluation of gastroretentive buoyant Sodium alginate microbeads of theophylline to increase the residence time in stomach and to modulate the release behaviour of the drug. Hydrophilic polymer Methocel K100M was used for its gel forming and release controlling properties. The effects of gas generating agent (sodium bicarbonate and calcium carbonate), curing time on drug release profile and floating properties were investigated. The floating bead formulations were prepared by dispersing theophylline together with calcium carbonate into a mixture of sodium alginate and hydroxypropyl methylcellulose solution (Methocel K100M) and then dripping the dispersion into an acidified solution of calcium chloride. Calcium alginate beads were formed, as alginate undergoes ionotropic gelation by calcium ions and carbon dioxide develops from the reaction of carbonate salts with acid. The evolving gas permeated through the alginate matrix,

leaving gas bubbles or pores, which provided the beads buoyancy. The prepared beads were evaluated for percent drug loading, drug entrapment efficiency, surface topography, buoyancy and *in vitro* release. Kinetic modeling of dissolution profiles revealed that the drug release mechanism was Fickian diffusion ($n < 0.5$) which was found to be governed by the concentration of polymer and gas generating agent.

Patil G.B *et al.,* 2009 Developed chitosan coated mucoadhesive multiparticulate drug delivery system for gliclazide. The purpose of this research work was to develop optimized and systematically evaluate performances of mucoadhesive microcapsules of antidiabetic drug gliclazide. Alginate microcapsules coated with mucoadhesive polymer chitosan were prepared by ionotropic gelation technique utilizing calcium chloride ($CaCl_2$) as a cross linking agent, to take the advantage of swelling and mucoadhesive property of alginate beads for improving the oral delivery of gliclazide. Depending upon the variability in the concentration of alginate, percentage of cross linking agent, time of curing, the factors like particle size, incorporation efficiency and release rate of microcapsules varies. The microcapsules obtained were discrete, spherical and free flowing. The microcapsules coated with mucoadhesive polymer chitosan exhibited good mucoadhesive property in the *in vitro* wash off test and also showed high percentage drug entrapment efficiency. The swelling behavior was strongly depends upon chitosan concentration. The *in vitro* release study indicates that the swelling is the main parameter in controlling the release rate from microcapsules.

Sunitha dahiya *et al.,* 2008 Prepared and evaluated oxytetracycline hydrochloride microbeads for delayed release. Oxytetracycline microbeads possessed average particle size range of 639.86 to 685.74 µm. *In vitro* drug release study was carried out in simulated gastric fluid for first 2 hr and simulated intestinal fluid for next 6 hr. Selected formulation was

coated using enteric polymer cellulose acetate phthalate to minimize burst drug release along with delayed drug release in intestinal medium.

Payam Khazaeli *et al.*, 2008 formulated ibuprofen beads by ionotropic gelation. Microencapsulation has become a common technique in the production of controlled release dosage forms. Many results have been reported, concerning the use of alginate beads as controlled release drug formulations. Alginate has a unique gel-forming property in the presence of multivalent cations, in an aqueous medium. Ibuprofen is an excellent analgesic and antipyretic, non-steroidal anti-inflammatory agent with a high therapeutic index. Formulation of ibuprofen in beads could reduce its gastric ulcerogenicity. Hence, in this study the formation of Ca-alginate ibuprofen beads, through ionotropic gelation has been investigated. For this purpose, different cross- linking agents including: Ca^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Sn^{2+} and Pb^{2+}, were used for bead preparation. Next, characterization of the beads, size distribution, encapsulation efficiency of ibuprofen within the beads, the bead swelling and the drug release kinetic were investigated. Results showed that only Ca ion is suitable for the formation of ibuprofen beads. A good swelling profile for beads in phosphate buffer (pH=7.4) and the lack of swelling in hydrochloric acid (pH= 1.2), show the suitable nature of the beads. In addition, formulation of Na-alginate (2%) and Ca-chloride (2%) beads resulted in an encapsulation efficacy of around 90%. The drug release studies showed a rapid and complete ibuprofen release from the beads, especially those prepared from Na-alginate (2%) and Ca-chloride (2%), in phosphate buffer medium. However, no detectable drug release was observed within the acidic medium. In conclusion, ibuprofen is capable of being microencapsulated as a bead formulation, with suitable properties and release profile.

Mutasem O. Taha *et al.,* 2008 developed Sodium lauryl sulfate impedes drug release from zinc-crosslinked alginate beads: Switching from enteric coating release into biphasic profiles. The aim of this research is to investigate the effects of sodium lauryl sulfate (SLS) on ionotropically cross-linked alginate beads. Different levels of SLS were mixed with sodium alginate and chlorpheniramine maleate (as loaded model drug). The resulting viscous solutions were dropped onto aqueous solutions of zinc or calcium ions for ionotropic curing. The generated beads were assessed by their drug releasing profiles, infrared and differential scanning colorimetery (DSC) traits. SLS was found to exert profound concentration-dependent impacts on the characteristics of zinc-crosslinked alginate beads such that moderate modifications in the levels of SLS switched drug release from enteric coating-like behavior to a biphasic release modifiable to sustained-release by the addition of minute amounts of xanthan gum. Calcium cross-linking failed to reproduce the same behavior, probably due to the mainly ionic nature of calcium–carboxylate bonds compared to the coordinate character of their zinc–carboxylate counterparts. Apparently, moderate levels of SLS repel water penetration into the beads, and therefore minimize chlorpheniramine release. However, higher SLS levels seem to discourage polymeric cross-linking and therefore allow biphasic drug release.

Han M.R. *et al.,* 2007 Studied pH dependent release property of alginate beads containing calcium carbonate particles. Alginate bead containing calcium carbonate particle were prepared by dropping the suspension of alginate/calcium carbonate (4/1, w/w) into aqueous solution of $CaCl_2$ (0.1M). The pH-dependent release property of the bead was observed for 12 h using blue dextran as a model drug. The release increased up to 4 h in a saturation manner. When no calcium carbonate was contained, the release exhibited no marked variation with pH and the values were 27–39%. On the other hand, in case calcium carbonate

was included in the matrix of alginate beads, intensive release (40–50%) was achieved in acidic and neutral conditions and the degrees of release were suppressed in alkali conditions and the values was 20%. The pH-sensitive release property is possibly because the particles of calcium carbonate embedded in the matrix of beads were leached out in acidic and neutral conditions, leaving cavities in the matrix. The cavities are likely to be main pathways for the release of blue dextran.

Marccus D Darrabie *et al.,* 2007 studied the effect of alginate composition and gelling cation on microbead swelling. The purpose of this study was to determine the roles of alginate composition and gelling cations on bead swelling, which affects its durability. Using a 2-channel droplet generator, microspheres were generated with 1.5% solutions of low viscosity high-mannuronic acid (LVM), medium viscosity high-mannuronic acid (MVM), low viscosity high-guluronic acid (LVG) and medium viscosity high-guluronic acid (MVG) alginate. They were gelled by cross-linking with 1.1% solution of either $BaCl_2$ or $CaCl_2$. The diameters of the microbeads were measured and recorded on day 0. The microbeads were subsequently washed and incubated in saline at 37˚C for 2 weeks with size assessment every 2 days. The data were normalized by calculation of the percentage change from control (day 0) for all groups of microbeads. Diameters of all beads were between 550–700 microns on day 0. Viscosity had no effect on swelling of Ba^{++} and Ca^{++}-alginate microbeads. Ca^{++}-alginate microbeads were more prone to swelling than the corresponding Ba^{++}-alginate beads. High G-Ba^{++} beads had only a modest increase in size over time, in contrast to the high M-Ba^{++}. Alginate composition and the gelling cation have significant effects on bead swelling.

Yagnesh L. Patel *et al.,* 2006 studied the effect of drug Concentration and Curing Time on processing and Properties of Calcium Alginate Beads Containing Metronidazole by Response

surface Methodology. The purpose of present research work was to prepare calcium alginate beads containing water-soluble drug metronidazole using 3^2 factorial design, with drug concentration and curing time as variables. Curing time was kept as low as possible to improve entrapment with increasing drug concentration. Mostly the drugs which had been encapsulated were water insoluble to facilitate drug encapsulation; a characteristic drug release as whole process is aqueous based. Entrapment efficiency was in the range of 81% to 96% wt/wt, which decreased with decrease in polymer concentration and increase in curing time. The beads were spherical with size range between 1.4 and 1.9 mm. Scanning electron microscope (SEM) photomicrographs revealed increase in the leaching of drug crystals with increased curing time and high drug concentrations. In acidic environment, the swelling ratio was 200% in 30 minutes, but in basic medium, it increased to a maximum of 1400% within 120 minutes. In acidic medium, the swelling and drug release properties were influenced by drug solubility, whereas in phosphate buffer these properties were governed by the gelling of polymer and exhibited curvilinear and quadratic functions of both the variables, respectively.

George Pasparakis and Nikolaos Bouropoulus 2005 Reported swelling studies and in vitro release of verapamil from calcium alginate and chitosan-calcium alginate beads. The aim of the present work was to investigate the swelling behavior and the in vitro release of the antihypertensive drug verapamil hydrochloride from calcium alginate and chitosan treated calcium alginate beads. Calcium–alginate beads, chitosan-coated alginate beads and alginate–chitosan mixed beads were synthesized and their morphology was investigated by scanning electron microscopy. The swelling ability of the beads in different media was found to be dependent on the presence of the polyelectrolyte complex between alginate and chitosan, the pH of the aqueous media and the initial physical state of the beads. The results revealed that the encapsulation of verapamil in both calcium–alginate and calcium alginate–chitosan mixed

beads exceeded 80%. Considering the in vitro stability of verapamil encapsulating beads, 70% of the drug released from wet and dry plain calcium alginate beads within 1 and 3 h, respectively. The presence of chitosan was found to retard significantly the release from wet beads. However, in the case of dry beads the presence of chitosan had no significant effect on the initial release stage and significantly increased the release on the later stage. The results were analyzed by using a semi-empirical equation and it was found that the drug release mechanisms were either "anomalous transport" or "case-II transport".

Eng-Seng Chan *et al.,* 2005 studied the effect of formulation of alginate beads on their mechanical behavior and stiffness. The aim of this work was to determine the effect of formulation of alginate beads on their mechanical behavior and stiffness when compressed at high speed. The alginate beads were formulated using different types and concentrations of alginate and gelling cations and was produced using an extrusion dripping method. Single wet beads were compressed at a speed of 40 mm/min, and their elastic limits were investigated, and the corresponding force versus displacement data was obtained. The Young's moduli of the beads were determined from the force versus displacement data using the Hertz's contact mechanics theory. The alginate beads were found to exhibit plastic behavior when they were compressed beyond 50% with the exception of copper–alginate beads for which yield occured at lower deformation. Alginate beads made of higher guluronic acid contents and gelling cations of higher chemical affinity were found to have greater stiffness. Increasing the concentration of alginate and gelling ions also generated a similar effect. At such a compression speed, the values of Young's modulus of the beads were found to being the range between 250 and 900 kPa depending on the bead formulation.

Pornsak Sriamornsak *et al.,* 2005 studied the effect of acidic medium on swelling and release behaviors of chitosan-reinforced calcium pectinate gel beads. Chitosan-reinforced

calcium pectinate (ChCP) gel beads were prepared by ionotropic gelation method. The swelling of ChCP gel beads and release behavior of indomethacin from the beads were investigated and compared with conventional calcium pectinate (CP) gel beads. The factors, such as molecular weight of chitosan, concentration of chitosan, and release medium, which can have a significant effect on the swelling and release behaviors from the beads, were discussed in this study. The mechanical test showed that the ChCP beads have slightly higher strength than that of CP beads. The swelling index of the ChCP beads in acidic medium was much lower than that in neutral medium. The release of indomethacin from ChCP beads under conditions mimicking intestinal transit were evaluated in pH 7.4 Tris buffer. The acid pretreatment caused a faster drug release from ChCP beads. The less swelling in acidic medium and faster drug release of acid-pretreated ChCP beads may be due to the dissolution of chitosan from the beads in acidic medium, as no fluorescence signal was seen at the shell of the beads. The results suggested that the acid, which essentially found in stomach, influenced the swelling and release behaviors of ChCP beads.

Anil K. Anal and Willem F. Stevens 2005 developed Chitosan–alginate multilayer beads for controlledrelease of ampicillin. The aim of this study is to develop multilayer beads with improved properties for controlled delivery of the antibiotic ampicillin. Ionotropic gelation was applied to prepare single and multilayer beads using various combinations of chitosan and Ca^{2+} as cationic components and alginate and polyphosphate as anions. Beads prepared with higher concentrations of chitosan entrapped more ampicillin. During incubation in simulated gastric fluid, the beads swelled and started to float but did not show any sign of erosion. Single layer chitosan–alginate beads released 70% of the drug within 4 h. Multilayer beads released only 20–30% in the same period of time. During subsequent incubation in simulated intestinal fluid, both single and multilayer beads continued to release drug. At least

part of this release is due to disintegration of the beads. The rate of release both in gastric and intestinal fluid and the kinetics of disintegration in intestinal fluid can be controlled by changing the chitosan concentration in the coagulation fluid. The release of the drug can also be controlled by the degree of cross-linking using polyphosphate. Cross-linked multilayer beads were prepared that released only 40% of the entrapped drug during 24 h. It is concluded that chitosan–alginate multilayer beads, cross-linked with polyphosphate offer an opportunity for controlled gastrointestinal passage of compounds with low molecular weight like ampicillin.

Ma X.J *et al.*, 2002 Characterized the structure and diffusion behavior of Ca-alginate beads prepared with external or internal calcium sources. Ca-alginate beads were prepared with either external or internal calcium sources. The structures of both beads were investigated with the aid of scanning electron microscopy (SEM) and confocal microscopy. It was shown that the beads with internal calcium source had a looser structure and bigger pore size than those with external calcium source. The attempts to interpret the difference were carried out by determining the Ca content within the beads at various times, which indicated that it was the different gelation mechanisms that caused the difference of structures of both beads. Furthermore, it was also found that the diffusion rate of haemoglobin (Hb) within the beads with an internal calcium source was faster than that of the beads with an external one, which was consistent with the observation of their structures.

L.Y.Lim and Lucy S.C.Wan 1997 Studied propanolol hydrochloride binding in calcium alginate beads. The interaction of propranolol hydrochloride with alginate molecular chains in calcium alginate beads was investigated. The drug was either incorporated into formed calcium alginate gel beads or incorporated simultaneously with the gelation of alginate beads

by Ca^{2+}. Beads produced by the former method had a higher drug content and lower Ca^{2+} level compared to those prepared by the latter method. The extent of drug binding to the alginate molecules increased with decreasing Ca^{2+} levels in the beads, indicating that propranolol and Ca^{2+} shared common binding sites in the alginate chains. SEM appearance of the beads and the morphology of the alginate polymer in the beads were affected by the amounts of both propranolol and Ca^{2+} in the beads. Differential scanning calorimetry (DSC) analyses showed that the formation of the calcium alginate gel structure was impeded in the presence of propranolol molecules.

CHAPTER IV

AIM OF THE WORK

Candesartan cilexetil is an antihypertensive agent used in the treatment of hypertension and heart failure. It used in the range of 4-32mg 1-2 times a day.

The bioavailability of candesartan cilexetil is approximately 15 % have requires frequent administration of dose 4 – 32 mg, 1-2 times daily.

The ultimate objective of microparticulate delivery system is to control and extend the release of the active ingredient from the coated particles without attempting to modify the normal biofate of the active molecules in the body after administration and absorption.

Mucoadhesive is a topic of current interest in the design of drug delivery systems to prolong the residence time of the dosage form at the site of application or absorption and to facilitate intimate contact of the dosage form with the underlying absorption surface to improve and enhance the bioavailability of drug.

The mucoadhesive microbeads have advantages like efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site.

The purpose of present investigation is to develop alginate mucoadhesive beads with different crosslinking agents and mucoadhesive polymer like chitosan with drug candesartan cilexetil by ionotrophic gelation process.

CHAPTER V

PLAN OF WORK

Part 1

- Determination of λ_{max} for candesartan cilexetil
- Calibration curve for the drug in buffer P^H 1.2 and P^H6.5.

Part 2

Preformulation studies

- Ftir studies
- Dsc studies

Part 3

- Formulation of candesartan cilexetil microbeads by ionotrophic gelation method.

Part 4

- Determination of production yield of microbeads.
- Determination of particle size by sico image analyser.
- Determination of swelling index
- Determination of drug entrapment efficiency.

Part 5

- *In Vitro* release studies of candesartan cilexetil microbeads in two buffer media viz., P^H 1.2 and P^H 6.5 in USP dissolution apparatus type II.

Part 6

- Determination of mucoadhesive property of selected formulations by *in vitro* wash off test.

Part 7

- Morphologic study for best formulation using scanning electron microscopy.

CHAPTER VI

MATERIALS AND EQUIPMENTS

MATERIALS	DISTRIBUTORS
Candesartan Cilexetil	Ranbaxy Laboratories, Gurgaon.
Sodium Alginate	Loba chemie, Mumbai.
Chitosan	Hi Media Laboratories Pvt Limited, Mumbai.
Zinc Chloride	Nice Chemical Pvt Limited, kochi.
Calcium Chloride	Nice Chemical Pvt Limited, kochi.
Barium Chloride	Spectrum Reagents And Chemicals Pvt Limited, kochi.
Lead Nitrate	Chemico Laboratories (P) Ltd, Mumbai.
Pectin	Leo Chem, Bengaluru.
Xanthan Gum	Nice Chemical Pvt Limited, kochi.
Methanol	Astron Chemicals,Ahmedabad.
Iso Propyl alcohol	Qualigens Fine Chemicals.
Glacial acetic acid	Omega laboratory chemicals,Bangalore.
Eudragit L 100	Orchid Health Care,Chennai.
Talc	Nice chemicals,kochi.
Sodium Hydroxide Pellets	Central Drug House,New Delhi.
Sodium Di Hydrogen Orthophosphate	High Purity Laboratory Chemicals PVT LTD, Mumbai.
PEG 6000	S.D. Fine Chem Ltd , Mumbai.

EQUIPMENTS	SUPPLIERS
Electronic Weighing Balance,	A&D Company, Japan.
Image Analyser	Leica, Germany
Hot Air Oven	Sico, India.
Dissolution Apparatus	Lab India Disso apparatus 2000, India.
UV- Visible Spectrophotometer	Schimazdu UV-1700, Japan.
Vacuum oven chamber	Rands instruments, India.
Magnetic Stirrer	M.C.Dalal, Chennai.
Scanning Electron Microscope	DSC 200 TA Instrument , USA
FT -IR	Perkin elmer, Germany.
Differential Scanning Calorimeter	DSC200 TA instuments, USA.
Disintegration Apparatus	Rolex, India.

CHAPTER VII

DRUG PROFILE

Candesartan Cilexetil

Molecular formula:

$C_{33}H_{34}N_6O_6$

Molecular weight:

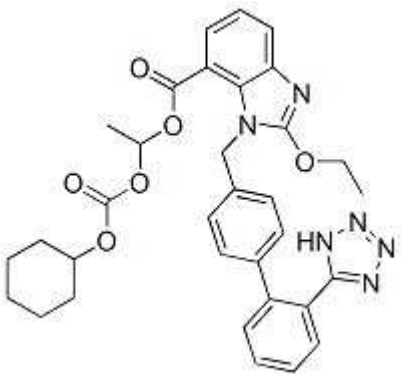
610.67

Description:

Candesartan cilexetil(atacand), a prodrug, is hydrolyzed to candesartan during absorption from the gastrointestinal tract. Candesartan is a selective AT_1 subtype angiotensin II receptor antagonist.

Candesartan cilexetil, a non peptide, is chemically described as (±)-1-Hydroxyl 2-ethoxy-l-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl) benzyl]-7-benzimidazolecarboxylate, cyclohexyl carbonate (ester).

Structure:



Physicochemical properties:

Candesartan cilexetil is a white to off-white powder. The solubility in benzyl alcohol is 205 g/l and the solubility in water is $<5x10^{-5}$ g/L.

Clinical pharmacology:

Mechanism of action:

Angiotensin II is formed from angiotensin I in a reaction catalyzed by angiotensin-converting enzyme (ACE, kininase II). Angiotensin II is the principal pressor agent of the renin-angiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation, and renal reabsorption of sodium. Candesartan blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT_1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland. Its action is, therefore, independent of the pathways for angiotensin II synthesis.

There is also an AT_2 receptor found in many tissues, but AT_2 is not known to be associated with cardiovascular homeostasis. Candesartan has much greater affinity (> 10,000-fold) for the AT_1 receptor than for the AT_2 receptor.

Blockade of the renin-angiotensin system with ACE inhibitors, which inhibit the biosynthesis of angiotensin II from angiotensin I, is widely used in the treatment of hypertension. ACE inhibitors also inhibit the degradation of bradykinin, a reaction also catalyzed by ACE. Because candesartan does not inhibit ACE (kininase II), it does not affect the response to bradykinin. Whether this difference has clinical relevance is not yet known. Candesartan does not bind to or block other hormone receptors or ion channels known to be important in cardiovascular regulation.

Blockade of the angiotensin II receptor inhibits the negative regulatory feedback of angiotensin II on renin secretion, but the resulting increased plasma renin activity and angiotensin II circulating levels do not overcome the effect of candesartan on blood pressure.

Pharmacokinetics:

General

Candesartan cilexetil is rapidly and completely bioactivated by ester hydrolysis during absorption from the gastrointestinal tract to candesartan, a selective AT1 subtype angiotensin II receptor antagonist.

Candesartan is mainly excreted unchanged in urine and feces (via bile). It undergoes minor hepatic metabolism by O-deethylation to an inactive metabolite. The elimination half-life of candesartan is approximately 9 hours. After single and repeated administration, the pharmacokinetics of candesartan are linear for oral doses up to 32 mg of candesartan cilexetil. Candesartan and its inactive metabolite do not accumulate in serum upon repeated once daily dosing.

Following administration of candesartan cilexetil, the absolute bioavailability of candesartan was estimated to be 15%. After tablet ingestion, the peak serum concentration (Cmax) is reached after 3-4 hours. Food with a high-fat content does not affect the bioavailability of candesartan after candesartan cilexetil administration.

Metabolism and Excretion

Total plasma clearance of candesartan is 0.37 mL/min/kg, with a renal clearance of 0.19 mL/min/kg. When candesartan is administered orally, about 26% of the dose is excreted unchanged in urine.

Following an oral dose of 14C-labeled candesartan cilexetil, approximately 33% of radioactivity is recovered in urine and approximately 67% in feces. Following an intravenous dose of 14C-labeled candesartan approximately 59% of radioactivity is recovered in urine and approximately 36% in feces. Biliary excretion contributes to the elimination of candesartan.

Distribution

The volume of distribution of candesartan is 0.13 L/kg. Candesartan is highly bound to plasma proteins (>99%) and does not penetrate red blood cells. The protein binding is constant at candesartan plasma concentrations well above the range achieved with recommended doses. In rats, it has been demonstrated that candesartan crosses the blood-brain barrier poorly, if at all. It has also been demonstrated in rats that candesartan passes across the placental barrier and is distributed in the fetus.

Special Populations

Pediatric:

The pharmacokinetics of candesartan cilexetil have not been investigated in patients <18years of age.

Geriatric and Gender:

The pharmacokinetics of candesartan has been studied in the elderly (≥65 years), and in both sexes. The plasma concentration of candesartan was higher in the elderly (Cmax was approximately 50% higher and AUC was approximately 80% higher) compared to younger subjects administered the same dose. The pharmacokinetics of candesartan were linear in the elderly, and candesartan and its inactive metabolite did not accumulate in the serum of these subjects upon repeated, once daily administration. No initial dosage adjustment is necessary. There is no difference in the pharmacokinetics of candesartan between male and female subjects.

Renal Insufficiency:

In hypertensive patients with renal insufficiency, serum concentrations of candesartan were elevated. After repeated dosing, the AUC and Cmax were approximately doubled in patients with severe renal impairment (creatinine clearance <30 mL/min/1.73m2) compared to patients with normal kidney function. The pharmacokinetics of candesartan in hypertensive patients undergoing hemodialysis are similar to those in hypertensive patients

with severe renal impairment. Candesartan cannot be removed by hemodialysis. No initial dosage adjustment is necessary in patients with renal insufficiency.

Hepatic Insufficiency:

No differences in the pharmacokinetics of candesartan were observed in patients with mild to moderate chronic liver disease. The pharmacokinetics after candesartan cilexetil administration has not been investigated in patients with severe hepatic insufficiency. No initial dosage adjustment is necessary in patients with mild hepatic disease.

S.No	Drug	Candesartan cilexetil
1.	Elimination half life	9hr
2.	Protein binding	99.5%
3.	Daily dose	4-32 mg
4.	No of doses per day	2
5.	Bioavailability	15%
6.	T_{max}	2-5 hr
7.	Clearance(ml/min)	26
8.	C_{max}	100ng/mL
9.	$T_{1/2}$	3-4 hr

INDICATIONS:

Candesartan cilexetil is indicated for the treatment of heart failure in patients with left-ventricular systolic dysfunction (ejection fraction ≤40%) to reduce cardiovascular death and to reduce heart failure hospitalizations. Candesartan cilexetil also has an added effect on these outcomes when used with an ACE inhibitor.

Candesartan cilexetil is indicated for the treatment of hypertension in adults and children 1 to <17 years of age. It may be used alone or in combination with other antihypertensive agents. This fixed dose combination is not indicated for initial therapy.

Occasionally, symptomatic hypertension has occurred after administration of candesartan cilexetil.

When pregnancy is detected, candesartan cilexetil should be discontinued because it can cause fetal and neonatal morbidity.

DRUG INTERACTIONS:

Wafarin

When candesartan cilexetil was administered at 16mg once daily under steady state conditions, no pharmacodynamic effect on prothrombin time was demonstrated in subjects stabilized on warfarin.

Digoxin

Combination treatment with candesartan cilexetil and digoxin in healthy volunteers had no effect on AUC or Cmax values for digoxin compared to digoxin alone. Similarly, combination treatment had no effect on AUC or Cmax values for candesartan compared to candesartan cilexetil alone.

Other

No significant drug interactions have been reported with glyburide, nifedipine or oral contraceptives co-administered with candesartan cilexetil to healthy volunteers. While there is no clinically relevant interaction between candesartan and enalapril, patients with renal impairment showed a higher exposure to both drugs. This is consistent with known pharmacokinetics of these two compounds.

OVER DOSAGE:

The most likely manifestations of overdosage would be hypotension, dizziness and tachycardia; bradycardia could occur from reflex parasympathetic (vagal) stimulation. In case reports detailing overdosage (up to 672 mg candesartan cilexetil) patient recovery was uneventful.

If symptomatic hypotension should occur, supportive treatment should be instituted and vital signs monitored. The patient should be placed supine with the legs elevated. If this is not sufficient, plasma volume should be increased by infusion of, for example, isotonic saline solution. Sympathomimetic drugs may also be administered if the above-mentioned measures are not sufficient. Candesartan cilexetil is not removed from the plasma by hemodialysis.

DOSAGE AND ADMINISTRATION:

Hypertension

Initiation of therapy requires consideration of recent antihypertensive treatment, the extent of blood pressure elevation, salt restriction, and other pertinent clinical factors. The dosage of other antihypertensive agents used with candesartan cilexetil may need to be adjusted. Blood pressure response is dose related over the range of 4 to 32 mg.

The recommended initial dose of candesartan cilexetil is 16 mg, once daily when used as monotherapy. Total daily doses of candesartan cilexetil should range from 8 to 32 mg. Doses higher than 32 mg do not appear to have a greater effect on blood pressure reduction, and there is relatively little experience with such doses. Most of the antihypertensive effect is present within 2 weeks and the maximal blood pressure reduction is generally obtained within 4 weeks. For patients with possible depletion of intravascular volume (e.g. patients treated with diuretics, particularly those with impaired renal function) consideration should be given to administration of a lower dose. If blood pressure is not controlled by candesartan cilexetil alone, candesartan cilexetil may be used together with a thiazide diuretic.

Heart failure:

The usual recommended initial dose for treating heart failure is 4 mg once daily. The target dose is 32 mg once daily which is achieved by doubling the dose at approximately 2 week intervals, as tolerated by the patient. ATACAND can be administered with other heart

failure treatments including ACE inhibitors, beta-blockers, diuretics, digoxin, and/or spironolactone.

No initial dose adjustment is necessary for elderly patients or in patients with renal or hepatic impairment.

SIDE EFFECTS:

The most common side effects of candesartan include headache, dizziness, fatigue, abdominal discomfort, diarrhea, and upper respiratory infections. Patients may also experience hyperkalemia, impotence, reduced renal function, and allergic reactions. Rhabdomyolysis (inflammation and destruction of muscle) and angioedema (swelling of soft tissues including those of the throat and larynx) are rare but serious side effects of candesartan (www.drugs.com).

CHAPTER VIII

EXCIPIENTS PROFILE

CHITOSAN

Chitosan is the term applied to deacetylated chitins in various stages of deacetylation and depolarization and it is therefore not easily defined in terms of its exact chemical composition. Partial deacetylation of chitin results in the production of chitosan which is a polysaccharide comprising copolymers of glucosamine and n-acetylglucosamine.

MOLECULAR WEIGHT

Chitosan is commercially available in several types and grades that vary in molecular weight between 10,000 and 1,000,000 and vary in degree of acetylation and viscosity.

SYNONYMS

2-amino-2 –deoxy-(1,4)-ß-d-glucopyranan; deacetylated chitin; deacetyl chitin; ß-1,4- amino 2-deoxy-d-glucopyranosamine

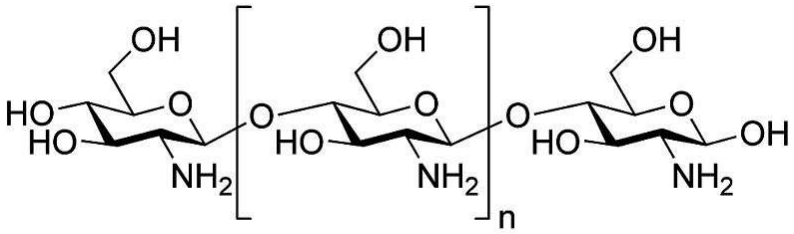
CHEMICAL NAME

poly-ß-(1,4)-2-amino-2-deoxy-d-glucose

DESCRIPTION

chitosan occurs as odorless,white or creamy whitepowder or flakes.fibre formation is quite common during precipitation and the chitosan may look ‘cotton like’.

STRUCTURAL FORMULA



FUNCTIONAL CATEGORY

Coating agent,disintegrant; film forming agent;mucoadhesive;tablet binder;viscosity-increasing agent

TYPICAL PROPERTIES:

Acidity

PH 4.0-6.0 (1%w/v aqeous solution)

Density

1.35-1.40 g/cm3

Glass transition temperature

203^{o}C

MOISTURE CONTENT

Chitosan absorbs moisture from the atmosphere ,the amount of water absorbed depending upon the initial moisture content and the temperature and relative humidity of surrounding air.

PARTICLE SIZE DISTRIBUTION

<30 mm

SOLUBILITY

Sparingly soluble in water, practically, insoluble in ethanol 95% and other organic solvents and neutral or alkali solutions at pH above 6.5.

INCOMPATIBILITY

Chitosan is incompatible with strong oxidizing agents.

SAFETY

Chitosan is investigated widely for use as an excipient in oral and other pharmaceutical formulation. It is biocompatible with both healthy and infected skin.chitosan has been shown to be biodegradable.

STABILITY AND STORAGE CONDITIONS:

Chitosan powder is a stable material at room temperature, although it is hygroscopic after drying. Chitosan should be stored in tightly closed comtainer in a cool, dry place and it should be stored at a temperature of 2-80c

APPLICATIONS IN PHARMACEUTICAL FORMULATION:

The suitability and performance of chitosan as a component of pharmaceutical formulations for drug delivery applications has been investigated in numerous studies. These include controlled drug delivery applications, used as a component of mucoadhesive dosage forms, rapid release dosage forms, improved peptide delivery.chitosan has been processed in to several pharmaceutical dosage forms, including gels, films, beads, microspheres, tablets and coating of liposomes.(Raymond *et al.,* 2006).

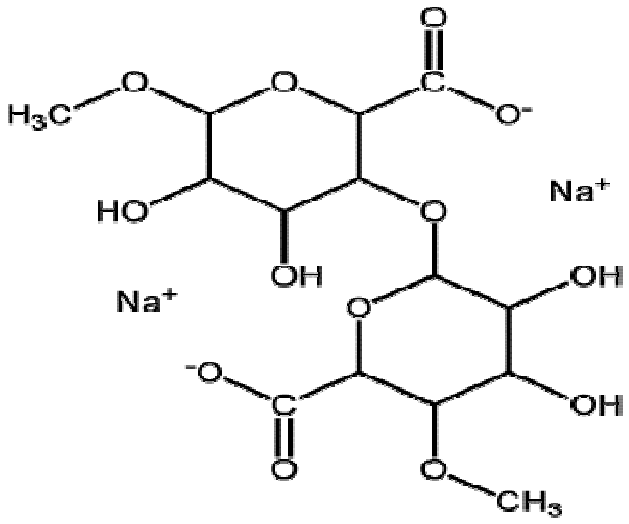
SODIUM ALGINATE

Sodium alginate consisting of sodium salt of alginic acid which is a mixture of polyuronic acids [$(C_6H_7O_6n$] composed of ß-d mannuronic acid and residue linked so that the carboxyl group of each unit is free while the aldehyde group is shielded by a glycosidic linkage.

EMPIRICAL FORMULA

$(C_6H_7O_6Na)_n$

STRUCTURAL FORMULA



DESCRIPTION

Sodium alginate occurs as a white or buff powder which is odourless and tasteless.sodium alginate produces aqeous solution that forms gel on the addition of small amount of soluble calcium salt.

GRADES

Various grades of sodium alginate are available yielding aqeous of varying viscosities within a range of 20 to 400 centipoises in 1% solution of 200c.

SOLUBILITY

Sodium alginate is very soluble in water forming a viscous colloidal solution practically it is inso.uble in alcohol,chloroform and ether and in hydroalcoholic solutions in which alcohol content is greater than 30% by weight.

STABILITY AND STORAGE CONDITIONS

Sodium alginate is hygroscopic,the moisture content at equilibrium is a function of relative humidity. Dry storage stability is excellent when the powder stored in a well closed container at temperature of 250c or less.

INCOMPATIBILITY

Depending on the concentration, sodium alginate is incompatible with phenolsand parabens.

USES

Alginic acid and alginates such as propylene glycol alginate and sodium alginate are used as suspending and thickening agents, aid in the preparation of water miscible pastes creams and gels. They may be used as stabilizers for water in oil emulsions and as binding aand disintegrating agents in tablets. Alginic acid and alginates are also employed as emulsifiers and stabilizers in food industry (Raymond *et al.,* 2006*)*.

XANTHAN GUM

EMPIRICAL FORMULA AND MOLECULAR WEIGHT

$(C_{35}H_{49}O_{29})n$

SYNONYMS

Corn sugar gum; E415; Keltrol; polysaccharide B-1459; Rhodigel; Vanzan NF; Xantural.

DESCRIPTION

Xanthan gum occurs as a cream- or white-colored, odorless,free-flowing, fine powder.

FUNCTIONAL CATEGORY

Stabilizing agent; suspending agent; viscosity-increasing agent.

TYPICAL PROPERTIES

Acidity/alkalinity: pH = 6.0–8.0 for a 1% w/v aqueous solution.

Freezing point: 08C for a 1% w/v aqueous solution.

Heat of combustion: 14.6 J/g (3.5 cal/g)

Melting point: chars at 2708C.

Particle size distribution: various grades with different particle sizes are available; for example, 100% less than 180 mm in size for Keltrol CG; 100% less than 75 mm in size for Keltrol CGF; 100% less than 250 mm, 95% less than 177 mm in size for Rhodigel; 100% less than 177 mm, 92% less than 74 mm in size for Rhodigel 200.

Refractive index: nD 20 = 1.333 for a 1% w/v aqueous solution.

Solubility: practically insoluble in ethanol and ether; soluble in cold or warm water.

Specific gravity: 1.600 at 258C

Viscosity (dynamic): 1200–1600 mPas (1200–1600 cP) for a 1% w/v aqueous solution at 258C.

STABILITY AND STORAGE CONDITIONS

Xanthan gum is a stable material. Aqueous solutions are stable over a wide pH range (pH 3–12), although they demonstrate maximum stability at pH 4–10 and temperatures of 10–60˚C.

Xanthan gum solutions of less than 1% w/v concentration may be adversely affected by higher than ambient temperatures: for example, viscosity is reduced. Solutions are also stable in the presence of enzymes, salts, acids, and bases. The bulk material should be stored in a well-closed container in a cool, dry place.

SAFETY

Xanthan gum is widely used in oral and topical pharmaceutical formulations, cosmetics, and food products and is generally regarded as nontoxic and nonirritant at the levels employed as a pharmaceutical excipient (Raymond *et al.,* 2006).

PECTIN

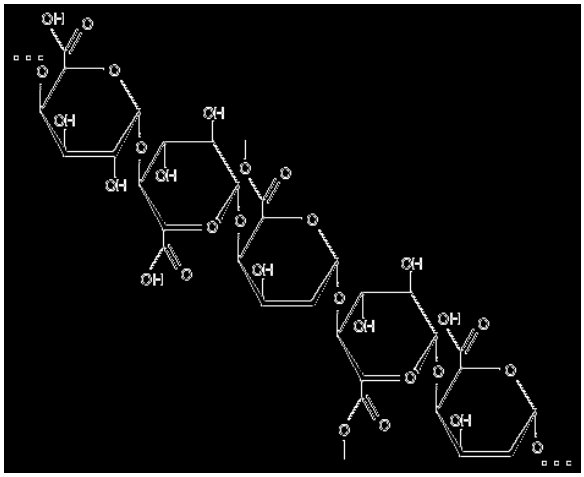
SYNONYMS

Citrus pectin; E440; methopectin; methyl pectin; methyl pectinate; mexpectin; pectina; pectinic acid.

EMPIRICAL FORMULA AND MOLECULAR WEIGHT

Pectin is a high-molecular-weight, carbohydrate-like plant constituent consisting primarily of chains of galacturonic acid units linked as 1,4-a-glucosides, with a molecular weight of 30 000–100 000.

STRUCTURAL FORMULA



FUNCTIONAL CATEGORY

Adsorbent; Emulsifying agent; Gelling agent; Thickening agent; Stabilizing agent.

DESCRIPTION

Pectin occurs as a coarse or fine, yellowish-white, odorless powder that has a mucilaginous taste.

TYPICAL PROPERTIES

Acidity/alkalinity: pH = 6.0–7.2

Solubility: soluble in water; insoluble in ethanol (95%) and other organic solvents.

Stability and Storage Conditions

Pectin is a nonreactive and stable material; it should be stored in a cool, dry place.

SAFETY

Pectin is used in oral pharmaceutical formulations and food products and is generally regarded as an essentially nontoxic and nonirritant material.

Low toxicity by the subcutaneous route has been reported (Raymond *et al.,* 2006).

CHAPTER IX

EXPERIMENTAL DETAILS

1. CALIBRATION FOR CANDESARTAN CILEXETIL:

1.1. Preparation of buffer solutions

Hydrochloric acid buffer pH 1.2

Take 50 ml of 0.2 M Potassium chloride in a 200 ml volumetric flask. 85 ml of 0.2 M Hydrochloric acid is added and made up to the volume with distilled water.

0.05m phosphate buffer pH 6.5

Dissolve 6.9g of sodium dihydrogen orthophosphate monohydrate in 900ml of distilled water. Adjust the pH using a 200mg/ml of NaOH. Dilute to 1000ml.

1.2. Calibration curve of candesartan cilexetil

Accurately Weighed 100 mg of Candesartan cilexetil is transferred to a 100 ml volumetric flask. It is dissolved in methanol and made up to the volume to get the primary stock solution. The secondary stock solution is prepared by diluting 10 ml of this solution to 100 ml volume with methanol i.e. 100 µg/ml concentration.

From the secondary stock solution, samples of 5 to 25 ml are pipetted out separately into separate volumetric flasks and diluted to 100 ml with buffer pH 1.2 to get concentrations of 5 to 25µg/ml respectively. And the same procedure is repeated with phosphate buffer of pH 6.5. The solutions are scanned in Ultra Violet (UV) Spectrophotometer to find out the λmax and the absorbance of the solutions is measured at the obtained λmax (257 nm).

The calibration graph is drawn by taking the concentration on X-axis and respective absorbance in Y-axis, to get a straight line as per the Beers law. This

standard curve is used to estimate the concentration of the drug released from the formulation during the in-vitro dissolution studies.

2. FORMULATION OF MUCOADHESIVE ALGINATE BEADS

The mucoadhesive alginate beads were prepared by ionotrophic gelation method as shown in Table 1. An aqeous solution of various concentrations of sodium alginate, xanthan gum and pectin (1%, 2%, 3%, 4%, and 5%) is prepared with vigorous stirring to form a clear solution. To this solution the drug candesartan cilexetil is added slowly and stirred continuously until a uniform dispersion is obtained. The dispersion is kept undisturbed for 30 minutes. The resultant bubble free, homogeneous dispersion is extruded into polyvalent ion solutions (200 ml) containing 1% chitosan using a hypodermic syringe with 21 gauge needle and stirred at 100rpm in magnetic stirrer. The gel beads are cured in gelation medium for 15 mins and then collected by decantation technique and the product thus separated is washed with acetone for two times and dried at room temperature for 24 hours (Anil.k.Anal et al ., 2005).

3. Preformulation studies

3.1 Fourier transformer infrared spectroscopic studies

FT-IR spectra (Spectrum RX-1 Perkin-Elmer,German) for the drug and various physical mixtures are obtained in a FT-IR spectroscopy in the transmission mode with the wave number region 4000-500cm^{-1}. KBr pellets are prepared by gently mixing 1mg sample powder with 100mg KBr. (S.K Swain *et al.,* 2011).

3.2. Differential scanning calorimeteric studies

DSC analysis (DSC200 TA instuments,USA) of the samples is carried out on a samples are heated under nitrogen atmosphere on an aluminium pan at a heating rate

of 10℃/min. Over the temperature range 5-300℃. DSC analysis is carried out under nitrogen gas flow of 20 lb/cm^2 (Anshu Sharma *et al.,* 2010).

4. Evaluation of mucoadhesive alginate beads

4.1. Percentage yield

The percentage yield of microcapsules of various batches are calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microbeads and percent yields is calculated as per the formula mentioned below.

$$\text{Percentage yield} = \frac{\text{Amount of dried microbeads}}{\text{Amount of drug+ Amount of polymer}} \times 100$$

4.2. Particle size measurement

Bead size measurement was characterized by using Leica image analyzer. Fifty completely dried alginate beads were taken and their size was measured (Eng-Seng Chan *et al.,* 2011).

4.3. Drug entrapment efficiency

Drug loaded microbeads (100 mg) are crushed in glass mortar and pestle and suspended in 100 ml of phosphate buffer (pH 6.5) solution and kept for 24hr. It is stirred for 5 minute and filtered by whatmann filter paper. Drug content in the filtrate is determined spectrophotometrically (S.P.Bhanja *et al.,* 2010).

Entrapment efficiency is calculated using the reported formula (Chowdary and Srinivasa, 2003).

$$\text{Entrapment efficiency} = \frac{\text{Actual \% drug content}}{\text{Theoretical \% drug content}} \times 100$$

4.4. Swelling studies

Swelling studies of chitosan-coated alginate beads are carried out in two media: simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF pH 6.5) for 12 hours. Accurately weighed amounts of beads (ranging from 2.5g) are immersed in 25 ml of SGF and SIF solution and at fixed time intervals the beads are separated from the medium using a stainless steel grid. Immediately, they are wiped gently with paper and weighed (M.Tavakol *et al.,* 2009). The swelling index of the beads with respect to time is calculated according to the formula:

$$\text{Swelling index} = \frac{Ws - Wi}{Wi} \times 100$$

Where *Wi* is the initial weight of beads

Ws is the weight of swollen beads

4.5. *In vitro* dissolution studies

In vitro dissolution studies was carried out in USP Type II (paddle) dissolution test apparatus using acid buffer pH 1.2(2 hours) and phosphate buffer pH 6.5(10 hours) as dissolution medium. For each batch 16 mg equivalent of alginate beads containing in enteric capsules are taken and subjected to dissolution studies. Volume of dissolution medium is 900 ml and bath temperature is maintained at 37±1°C throughout study. Paddle speed is adjusted to 50 rpm. An interval of 30 mins, 10 ml of sample is withdrawn with replacement of 10 ml fresh medium and analyzed for

candesartan cilexetil content by UV-Visible spectrophotometer at 257nm**.** (Patil Basawaraj *et.al.,* 2011)

4.6. Release kinetics

The dissolution data obtained are fit into various kinetic models, namely, zero order, first order, Higuchi, Hixson-Crowell, Korsmeyer-Peppas. This is to determine the mechanism of drug release.

Higuchi model (Katikaneni *et al.,* 1986) relates the relationship between quantity of drug released and the square root of time.

$$Q=K\ t^{1/2}$$

Quantity of drug released was plotted against square root of time. The Higuchi release constant k and R^2 value are extracted from the graph. The Higuchi constant reflects the design variables of the system. Hence drug release rate is proportional to the reciprocal of the square root of time.

For zero order, from the equation $C=K_0t$, drug concentration was plotted against time. The zero order rates constant k_0 and the regression line (R^2) values are also extracted from the graph.

For First order release kinetics, Log cumulative % drug remaining was plotted against time. The first order rate constant k_1 and the regression line value (R^2) are extracted from the graph.

For Hixson-Crowell release mechanism, Cube Root of initial amount of drug minus Cube Root of amount of drug released at time t was plotted against time in hour. Then the rate constant of release and the regression line value (R^2) are extracted from the graph.

To confirm the exact release mechanism operational the data are fitted according to Korsemeyer-Peppa's equation (Costa and Lobo, 2001; Sreenivas *et al.,* 2006;

Korsemeyer *et al.,*1983). This simple empirical equation is used to describe general solute release behaviour from controlled release polymer matrices.

$$mt/mT = k\ t^n.$$

Where

mt/*mT* is fraction of drug released,

k is kinetic constant, t is release time and

'n' is the diffusional exponent for drug release.

Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. The value of 'n' gives an indication of the release mechanism; when $n = 1$, the release rate is independent of time (zero-order) (case II transport), $n = 0.5$ for Fickian diffusion and when $0.5 < n < 1.0$, diffusion and non-Fickian transport are implicated.

Lastly, when $n > 1.0$ super case II transport is apparent. '*n*' is the slope value of log *mt*/*mT* versus log time curve (Tanwar *et al.,*2007). Here Log cumulative % drug release was plotted against Log time and the slope, 'n' and the regression line values (R^2) are extracted from the graph (Obitte N.C *et.al.,* 2010).

4.7. *In vitro* wash off test

The time taken for detachment of beads from sheep intestinal mucosa is measured in 0.1N hydrochloric acid (pH 1.2) and 0.05M phosphate buffer (ph 6.5). This is evaluated by an *in vitro* adhesion testing method, known as wash off method. A piece of sheep intestinal mucosa (2×2 cm) is mounted onto glass slide (3×1 inch) with cyanoacrylate glue. The beads (50 nos) are counted and spread over the wet rinsed tissue specimen and immediately thereafter the support is hung on the arm of a USP tablet disintegrating test machine. By operating the disintegration machine the

tissue specimen is given a slow regular up and down movement. The slides move up and down in the test fluid at 37 ± 0.5^0 C. The number of beads adhering to the tissue is counted at 2-hour intervals up to 12 hours (Lehr *et al* .,1991).

4.8. Scanning electron microscopic studies

The morphology and surface structure of beads are observed using SEM photographs taken with SEM analyser.

The beads are made conductive by sputtering thin coat of platinum under vacuum and then the images are recorded with at magnification of 25X. (Raghavendra V.Kulkarni *et.al.,* 2010).

CHAPTER X

RESULTS AND DISCUSSION

Mucoadhesive alginate beads of candesartan cilexetil were prepared by ionotrophic gelation method and various evaluation parameters were assessed, to obtain oral controlled release of candesartan cilexetil.

The prepared mucoadhesive alginate beads were then subjected to percentage yield, particle size determination, determination of drug content and entrapment efficiency, swelling studies*, in vitro* dissolution studies, *in vitro* wash off test, FT-IR studies, differential scanning calorimetry studies and scanning electron microscopy.

1. CALIBRATION OF CANDESARTAN CILEXETIL:

The λmax of candesartan cilexetil was determined by scanning the 10 µg/ml of the drug solution in buffer solution of pH1.2 and pH 6.5. It showed the λmax of 257nm in buffer solution of pH 1.2 and pH 6.5 respectively. A standard calibration curve for the drug was obtained by measuring absorbance at 257 nm and by plotting the graph of absorbance Vs concentration. The calibration plots of candesartan cilexetil were shown in Tables 2, 3 and Figure 2, 3 respectively. The linear correlation co-efficient was found to be $\gamma = 0.9994$ (for pH 1.2) and $\gamma = 0.9998$(for pH 6.5).

The standard plots of candesartan cilexetil are shown in Figure 1.

Estimation of drug content and in vitro release is based on this standard curve (Patil basavaraj *et al.,* 2011).

2. FORMULATION OF MUCOADHESIVE ALGINATE BEADS.

The mucoadhesive alginate beads were prepared according to the formulations shown in Table 1. The beads were prepared by extruding solution of sodium alginate, xanthan gum and pectin as droplets into a divalent cross linking agents such as calcium chloride, barium chloride, zinc chloride and lead nitrate was able to form uniform rigid beads. The needle size of 21G produced big size, rigid, uniform and porous beads (Anil.K.Anal *et al.,* 2005). The shapes of all the beads were almost spherical.

3. Preformulation studies

3.1. Fourier transformer infrared spectroscopic studies

To check the compatibility of drug with various polymers, IR spectra of drugs, polymers and combination of the drug and polymers were taken.

FTIR spectra of candesartan cilexetil, sodium alginate, xanthan gum, pectin and chitosan were recorded in KBr pellets and are presented in Figure 4.

The IR spectral analysis of candesartan cilexetil pure drug alone showed that principal peaks were observed at wave numbers 3411.67cm^{-1}, 2939.80cm^{-1}, 1753.03cm^{-1}, 1551.14cm^{-1} and 798.02cm^{-1}.

Further in the physical mixture of sodium alginate, xanthan gum, pectin, chitosan and candesartan cilexetil, the major peaks of were observed at 3412.95cm^{-1}, 2940.58cm^{-1}, 1714.69cm^{-1}, 1548.86cm^{-1} and 746.67cm^{-1} suggesting that there is no interaction between the polymers and drug used in the present study.

3.2. Differential scanning calorimetric studies

The DSC thermogram of pure drug and the different polymers were shown in the Figure 5. A sharp exothermic peak at about 173˚c was observed for pure candesartan cilexetil. It was observed that the large exothermic peak of pure drug was a bit smaller and shifted to 172.94 ˚c in physical mixture revealing its unchanged nature. This indicates that the drug has not undergone any chemical interaction with the polymer backbone.

4. Evaluation of mucoadhesive alginate beads.

4.1. Percentage yield

As shown in Table 4 the percentage yield of alginate beads prepared by ionotrophic gelation method were found to be between 76% and 94%. It was found that production yield of alginate beads prepared in calcium chloride was greater than barium chloride, zinc chloride and lead nitrate.

A significant decrease in the production yield was observed with increase of alginate concentration. The probable reason behind this may be due to high viscosity of the solution which decreases its syringeability resulting in blocking of needle and wastage of the drug polymer solution which ultimately decreased the production yield. The spherical shape of the beads in wet state was usually lost after drying especially for beads prepared with low concentration of sodium alginate and cross linking agent. With the increase in the concentration of sodium alginate the shape of the beads retained considerably (M.Tavakol *et al.,* 2009).

4.2. Particle size

By image analyzer it was found that the particle size was very well within a narrow size range between 1.073mm and 1.938mm. The mean particle size was different among the formulations as shown in Table 5. The effect of concentration of polymer on the size of beads formed were studied and it was found that there was increase in the average diameter of particles as there was an increase in the concentration of polymer.

The most important property of alginates is their ability to form gels by reaction with divalent cations such as ca^{2+} (Takka Acarturk, 1999; Aslani and Kennedy, 1996). The gelation and cross linking of the polymers are mainly occurred by exchange of sodium ions from the guluronic acids with the divalent cations and the stacking of these guluronic acids form the characteristic egg box spherical structure (Li *et al.,* 2007).

4.3. Drug entrapment efficiency:

The drug entrapment efficiency of all formulations was in the range between 70% and 90%. This is probably due to more firmness in the alginate-chitosan complex during gelation caused by increased ionic interactions between the carboxylate groups in the alginate and the protonated amine groups in the chitosan. (Anil.K.Anal *et al.,* 2005).The results of drug entrapment efficiencies were shown in table 6 and figure 6.

Drug entrapment efficiency of alginate beads increases with increase in concentration of polymers. The higher viscosity of the polymer solution at the highest polymer proportion would be expected to decrease the diffusion of the drug into the external phase which would result higher entrapment efficiency. This may be

attributed to the greater availability of active binding sites in polymeric chains and consequently the greater degree of cross linking as the quantity of alginate increased.

4.4. Swelling studies

Swelling of the dry beads is mainly attributed to the hydration of the hydrophilic groups of alginate and chitosan (Hoffman, 2002). In this case free water penetrates inside the beads in order to fill the inert pores among polymer chains, contributing to a greater swelling degree.

At pH 1.2, the swelling degree of the beads was limited due to the reduced chemical potential of the network resulted from protonation of carboxylic acid groups. The initial increase of swelling degree is mostly driven by counter ions which neutralize $-NH_3^+$ groups of polymer chain in the network. (Dolatabadi-Farhani, Vashegani-Farhani, & Mirzadeh, 2006).

All the formulations exhibited significant swelling rates when exposed to the slightly alkaline medium. This swelling mechanism in this case is related with the polyvalent ions and Na^+ exchange (Bajpai and Sharma 2004).

All beads began to swells presumably due to an increase in the electrostatic repulsive forces at a pH above the pka of the uronic acid groups on the alginate. (Phillipa Rayment *et al.,* 2004).

When the swelling degree of the formulations was compared, the lowest swelling ratio was obtained in pH 1.2. In pH 6.5, the alginate beads swelled and they were not broken in these pH values after 8 hours. These results suggest that alginate beads do not disintegrate in the stomach and thus resulted in release of candesartan cilexetil in intestinal fluids.

Table 7 and Fig 7 show the swelling behavior of beads, as a function of pH. The swelling degree of calcium- alginate beads was lower than that of zinc-alginate, barium-alginate, and lead-alginate beads. The increase in concentration of ca^{2+} ions in the gelation medium increases the availability of ca^{2+} ions which increase the number of interactions with coo^{-} groups present in alginate. This resulted in increased crosslinking density which hindered inward diffusion of swelling medium (Tonnesen and karlsen, 2002).

4.5. *In-vitro* dissolution studies:

The results of in-vitro drug release studies from the mucoadhesive alginate beads are shown in the Tables- 8A, 8B, 8C and 8D and in Figures 8A, 8B, 8C and 8D respectively.

The in-vitro dissolution studies of all formulations were carried out by USP type II method by using two different dissolution media pH 1.2 and pH 6.5. The studies were performed in all the formulations for 12 hours. Sampling was done every 30 minutes and absorbance was determined using UV spectrophotometer at 257 nm.

The beads did not show any drug release at pH 1.2 and it released the drug at pH 6.5. So it protected the release of drug from the acidic medium to minimize the side effects. Above pH 6.0 eudragit L 100 coating started to dissolve and exposed the alginate beads for drug release.

Effect of cross linking agents on drug release

The formulations F1, F2, F3, F4 and F5 were prepared with 2%, 4%, 6%, 8%, 10% barium chloride as cross linking agent showed the cumulative percentage of drug release 78.12%,76.07%,72.72%,71.17%,70.01% respectively at the end of 12 hours.

This shows that more sustained release was observed with increase in concentration of barium chloride.

The formulations F6, F7, F8, F9 and F10 were prepared with 2%, 4%, 6%, 8%, 10% zinc chloride as cross linking agent showed the cumulative percentage of drug release 80.01%,77.03%,75.59%,74.91%,73.40% respectively at the end of 12 hours. This shows that more sustained release was observed with increase in concentration of zinc chloride.

The formulations F11, F12, F13, F14 and F15 were prepared with 2%, 4%, 6%, 8%, 10% calcium chloride as cross linking agent showed the cumulative percentage of drug release 75.12%,73.71%,71.72%,69.62%,67.75% respectively at the end of 12 hours. This shows that more sustained release was observed with increase in concentration of calcium chloride.

The formulations F16, F17, F18, F19 and F20 were prepared with 2%, 4%, 6%, 8%, 10% lead nitrate as cross linking agent showed the cumulative percentage of drug release 81.08%,79.76%,78.42%,76.76% ,75.80% respectively at the end of 12 hours. This shows that more sustained release was observed with increase in concentration of lead nitrate.

Hence drug release is retarded in the following order.

Lead nitrate > zinc chloride > barium chloride> calcium chloride

The formulations F5, F10, F15 and F20 containing 10% barium chloride, 10% zinc chloride, 10% calcium chloride and 10% lead nitrate respectively showed more sustained release with the increase in percentage of cross linking agents. This indicates that the release rate is retarded due to increase in percentage of cross linking agents

because of strong bonds between sodium alginate and divalent ions.

Alginate able to form complex with divalent ions like zn^{2+}, ba^{2+}, ca^{2+} and pb^{2+}, although the association is stronger with calcium than barium, zinc and lead ions because calcium is more densely cross linked with alginate (Aslani and kennedy, 1996). This may be due to increase in Ca^{2+} ions with sodium alginate, interaction between Na^{+} and Ca^{2+} is increased, forming closer network which decreased the diffusion of drug outwards the alginate beads.

The batch containing sodium alginate-chitosan and calcium chloride showed sustained release of drug upto 12hr when compared with other cross linking agents. This may be due to stronger affinity of ca^{2+} ions towards alginate than other cross linking agents' results in resistance of the polyelectrolyte complex against drug diffusion and slower release of drug from alginate beads (Cheong Hian Guh *et al.,* 2012).

Increase in the concentration of calcium ions with sodium alginate permitting a higher extent of cross linking that led to stronger bead formation, providing greater resistance to drug diffusion from the alginate beads (Ostberg *et al.,* 1994).

Effect of sodium alginate on drug release:

As increasing the percentage of sodium alginate in formulation could led to a greater viscosity of the solution, and hence large drops needs to be dripped out of the needle. As a result, larger beads were formed and diffusivity decreased (Payam khazaeli *et al.,* 2008). As the polymer to the drug ratio was increased the extent of drug release decreases. The decrease in the rate and extent of the drug release is due to

the higher density of polymer matrix that results in increased diffusion pathlength through which the drug molecule have to traverse (J.A. Ko *et al.,* 2002).

4.6. Release kinetics

Further the drug releases were subjected for mathematical treatment to check whether the release is following first order kinetics or zero order kinetics. The correlation co efficient values are shown in Table 9.

The r values of zero order plots were between 0.981 to 0.986 and first order plot between 0.925 and 0.982. The r^2 values indicate all these formulations followed zero order kinetics.

The values of co efficient of correlation were found to be best fitted to korse meyer peppas and higuchi model. The R^2 values are closer to one in zero order kinetics, so it follows zero order kinetics.

The diffusional exponent, n, specifies the mechanism of release. For alginate beads, values of 'n' between 0.43 and 0.85 are an indication of both diffusion controlled drug release and swelling controlled drug release (anomalous transport or non-fickian diffusion). Values above 0.85 indicate case II transport which relate to polymer relaxation during gel swelling (Siepmann and peppas, 2001; Ritger peppas, 1987).

The release kinetics of best formulation is shown in Fig- 9.

4.7. *In vitro* wash off test:

The results of in vitro wash off test for selected formulations were shown in Table 10. Mucoadhesive property was studied on the selected formulations (F5, F10, F15 and F20) of alginate beads by *in vitro* wash off test method. The percentage of

alginate beads attached to the goat intestinal mucosa after 12 hours is shown in Table 10. The alginate beads prepared with calcium chloride-sodium alginate-1% chitosan showed good mucoadhesive property. The following stages may have occurred during mucoadhesion. Initially, an intimate contact i.e., (wetting) between the mucus gel and the swelling of mucoadhesive polymer, which makes the polymer strands to relax, this is followed by the penetration of the mucoadhesive polymer into the mucus gel network and finally the formation of secondary chemical bond between the mucus and the mucoadhesive polymer. It was found that the pH of the medium is important for the hydration, solubility and mucoadhesion of the polymer (Lehr *et al.,* 1991).

4.8. Scanning electron microscopy analysis

A SEM photograph of formulation (F-15), a single bead taken at 25X magnification, was shown in Figure 10. As seen from the Figure, the drug loaded alginate bead was almost of spherical in shape and have rough surface.

CHAPTER XI

SUMMARY AND CONCLUSION

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body and also to achieve and maintain the desired drug concentration.

Candesartan cilexetil is an antihypertensive agent used in the treatment of heart failure. The bioavailability of candesartan cilexetil is 15%.

An attempt is made to microencapsulate candesartan cilexetil by ionotropic gelation method with a view to prevent the gastric side effects and to achieve an oral controlled release of the drug.

The literature review discussed reveals the research work done to develop microparticulate drug delivery system containing anti-diabetic, anti-hypertensive and NSAIDS drugs by ionotrophic gelation method.

Polymers used in this method are sodium alginate, xanthan gum, pectin, barium chloride, zinc chloride, calcium chloride and lead nitrate as crosslinking agents and chitosan as mucoadhesive polymer.

In present study twenty formulations were formulated by using sodium alginate, pectin, xanthan gum and chitosan in various proportions.

All the formulations were subjected for evaluation. Results of preformulation studies, particle size, entrapment efficiency, swelling studies, *in vitro* dissolution study and *in vitro* wash off test have shown satisfactory results.

In vitro release study shows a retarded release with increase in percentage of sodium alginate, pectin and xanthan gum.

On the basis of release data and kinetic analysis F15 showed a good controlled release profile with maximum entrapment efficiency.

The release kinetics of formulations shows a good correlation of korsemeyer peppas with a good 'n' value. According to 'n' values obtained formulation following non fickian diffusion as release mechanism.

The selected formulation showed good mucoadhesive property by *in vitro* wash off test.

The surface study of F15 viewed through SEM shows an uniform matrix formulation with dense nature and low porosity.

Conclusion:

The mucoadhesive microbeads of candesartan cilexetil could be prepared by an ionotrophic gelation method. Among the four different cross linking agent's calcium chloride higher entrapment, particle size, good mucoadhesive property in the *in vitro* wash off test. Drug release was diffusion followed zero order kinetics. The results of our study suggest that mucoadhesive microbeads of candesartan cilexetil are suitable for oral controlled release.

REFERENCES

Adhiyaman Rajendran and Sanat Kumar Basu., 2009. Alginate-Chitosan Particulate System for Sustained Release of Nimodipine, Tropical J. Pharm. Res., 8(5): 433-440.

Alireza Mortazavi. S and John D. Smart., 1993. An investigation into the role of water movement and mucus gel dehydration in mucoadhesion. J.contr Rel., 25: 197-203.

Al-Musa S., Abu Fara D., Badwan A.A., 1998. Evaluation of parameters involved in preparation and release of drug loaded in crosslinked matrices of alginate. J. contr rel., 57(1999): 223-232.

Amit Kumar Nayak., Ruma Maji and Biswarup Das., 2010. Gastroretentive drug delivery systems: a review, Asian J. Pharm and Clin Res, 3(1); 2-10.

Amit Kumar Nayak., M. Saquib Hasnainb., Sarwar Begc., M. Intakhab Alamc., 2010. Mucoadhesive beads of gliclazide: Design, development and evaluation, Science Asia 36: 319–325.

Anil K. Anal., Willem F. Stevens., 2005. Chitosan–alginate multilayer beads for controlled release of ampicillin, Int. J. Pharm., 290, 45–54.

Anurag Sood and Ramesh Panchagnula., 1998. Drug release evaluation of diltiazem CR preparations, Int. J. Pharm, 95-107.

Ashok Kumar.A., Balakrishna.T., Rajiv Jash., T.E.G.K Murthy., Anil Kumar.A., and B. Sudheer., 2011 Formulation And Evaluation Of Mucoadhesive Microcapsules Of Metformin Hcl With Gum Karaya, Int. J. Pharm. & Pharm. Sci, 3(3): 150-155.

Bagyalakshmi J., Arun Raj R., and Ravi T K., 2011; Formulation, physical characterisation and *in-vitro* release studies of prednisolone alginate beads for colon targeting by ionotropic gelation, Int. J. comp. Pharm, 2(04) 1-3.

Bhanja S. B., Ellaiah P., Marth S. K., Murthy K.V.R., Panigrahi B., Das D. 2010. Preparation and *in-vitro* evaluation of mucoadhesive microcapsules of acyclovir, Int. J. Pharm. Res and Dev, Vol. 2(4) 907-923.

Bindu Madhavi. B., Ravinder Nath. A., David Banji, Ramalingam. R., Naga Madhu R.M., Arjun G., Sriharsha V., 2009. Formulation and evaluation of venlafaxine hcl enclosed in alginate microbeads prepared by iontophoretic gellation method Int. J. Pharm. Res. & Dev, vol 8 1-11.

Brahmankar D M and Sunil B Jaiswal., 2001. Biopharmaceutics and pharmacokinetics A treatise, 1st edition, New delhi; 337-341.

Cenk Aral., and Julide Akbug., 1998. Alternative approach to the preparation of chitosan beads, Int. J. Pharm: 9–15.

Cheong Hian Goh., Paul Wan Sia Heng., Lai Wah Chan., 2012. Cross-linker and non-gelling Na^+ effects on multi-functional alginate dressings. Carbohydrate polym, 87(2012) 1796– 1802.

Choudhury P. K., Panigrahi G. S., Pradhan K. K., Panda1 C. K., Pasa G. S., 2010. Design, development and evaluation of frusemide loaded micropellets prepared by ionotropic gelation method Int. J. Pharm.Tech. Res., 2(1):420-426.

Chowdary K.P.R and Sri ram murthy., 1998. Microencapsulation in pharmacy Indian drugs, 25(10): 389-402.

Anthony C Moffat., M David osselton and Brian widdop., Clarke analysis of drug and poisons, **2004**. 3rd edition, vol 2: 740-741.

Claus-Michael Lehr., Joke A. Bouwstra., Etienne H. Schach and Hans E. Junginger., 1992. *In vitro* evaluation of mucoadhesive properties of chitosan and some other natural polymers Int J. Pharm, 78: 43-48.

Dr. Hitesh Patel., Dr. Ritesh Patel., Dr. Girish Patel., 2010. Ionotropic Gelation Technique For Microencapsulation Of Antihypertensive Drug, Webmedcentral 1(10):1-10.

Felipe J.O. Varum,, Francisco Veig., Joao S. Sousa., Abdul W. Basit., 2010. An investigation into the role of mucus thickness on mucoadhesion in the gastrointestinal tract of pig. Eur J. Pharm Sci 40: 335–341.

Ferreira Almeida P, Almeida A.J. 2004. Cross-linked alginate–gelatine beads: A new matrix for controlled release of pindolol, J. Cont. Rel, 431– 439.

Flávia Chiva Carvalho., Marcos Luciano Bruschi., Raul Cesar Evangelista., Maria Palmira Daflon Gremiã., 2010. Mucoadhesive drug delivery systems, Braz. J. Pharm Sci, 46(1): 1-18.

George Pasparakis., Nikolaos Bouropoulos., 2006. Swelling studies and *in vitro* release of verapamil from calcium alginate and calcium alginate–chitosan beads, Int. J. Pharm, 34–42.

Govind S. Asane., Yamsani Madhusudan Rao., Jaykrishna H. Bhatt., Karimunnisa S. Shaikh., 2010. Optimization, Characterisation and Pharmacokinetic Studies of Mucoadhesive Oral Multiple Unit Systems of Ornidazole, Sci Pharm, 79: 181–196.

Hagerstrom, Helene, 2003, Polymer Gels as Pharmaceutical Dosage Forms, Compreh Summ of Uppsala.

Han M. R., Kwon M. C., Lee H. Y., Kim J. C., Kim J. D., Yoo S. K., Sin I. S., Kim S. M., 2007. pH-dependent release property of alginate beads containing calcium carbonate particles. J. Microencapsulation, 24(8): 787‑796.

Harshad Parmar., Sunil Bakliwal., Nayan Gujarathi., Bhushan Rane., Sunil Pawar., 2011. Formulation, Optimization And *In Vitro* Characterization Of Mucoadhesive Microparticle, Int. J. Pharm. & Bio Arch, 2(3): 880-886.

Hemanta Kumar Sharma., Babita Sarangi., Siba Prasad Pradhan., 2010. Preparation and *in-vitro* evaluation of mucoadhesive microbeads containing Timolol Maleate using mucoadhesive substances of *Dillenia indica L*, Arch Pharm. Sci & Res.,1 (2) 181 -188.

Herbert A. Lieberman., Leon lachman and Joseph B. Schwartz., Pharmaceutical dosage forms IInd edition, 1(7).

Jayvadan Patel., Darshna Patel., Jignyasha Raval., 2010. Formulation and Evaluation of Propranolol Hydrochloride-Loaded Carbopol-934P/Ethyl Cellulose Mucoadhesive Microspheres, Iran J. Pharm. Res., 9 (3): 221-232.

John D. Smart., 2005. The basics and underlying mechanisms of mucoadhesion. Adv Drug Del Rev., 57: 1556– 1568.

Karthikeyan Kesavan., Gopalnath., Jayanta K. Pandit., 2010. Sodium Alginate BasedMucoadhesive System for Gatifloxacin and Its *In Vitro* Antibacterial Activity. Sci pharm., 78: 941–957.

Ko J.A., Park H.J., Hwang S.J., Park J.B., Lee J.S., 2002. Preparation and characterization of chitosan microparticles intended for controlled drug delivery. Int J. Pharm 249: 165-174.

Korsemeyer R W., Gurny R., Doelkar E M., Buri P., Peppas N.A., 1983. Mechanism of solute release from porous hydrophilic polymers, Int. J. Pharm.1, 25-35.

Kundlik M. Girhepunje, Krishnapillai, Ranju S. Pal, Hitesh B. Gevariya, N. Thirumoorthy., 2010 Celecoxib loaded microbeads. A targeted drug delivery for colorectal cancer, Int. J. Cur Pharm Res, 2(1): 46-55.

Liu X. D., Yu W.Y., Zhang Y., Xue W.M., Yu W.T., Xiong Y., Ma X. J., Chen Y. and Yuan Q.,2002 Characterization of structure and diffusion behaviour of Ca-alginate beads prepared with external or internal calcium sources, J. Microencapsulation, 19(6): 775-782.

Madan Jyotsana., Banode Sagar., Dangi Mahesh., 2010. Mucosal drug delivery system, Int. J. Res. Ayur. Pharm. 1(1): 63-70.

Marcus D. Darrabie., William F. Kendall., Emmanuel C. Opara., 2005. Effect of alginate composition and gelling cation on microbead swelling. J. Microencapsulation; 23(6): 613–621.

Margret Chandira., Sachin., Debjit Bhowmik., B. Jayakar., 2009. Formulation and evaluation of mucoadhesive oral tablet of clarithromycin, T. Pharm. Res, 2(1); 30-42.

Md Masud Morshed., Jewel Mallick., Aninda Kumar Nath., Md Zia Uddin., Mycal Dutta., Md Akbar Hossain and Md Hassan Kawsar., 2012. Effect of Barium Chloride as A Cross

Linking Agent on the Sodium Alginate Based Diclofenac Sodium Beads. Bang Pharm. Jour. 15(1): 53-57.

Mohammed G Ahmed., Satish K BP., Kiran K GB., 2010. Formulation and evaluation of gastric-mucoadhesive drug delivery systems of captopril, J. Cur. Pharm. Res. 2(1): 26-32.

Mohd Yasir., Meenakshi Bajpai., Arundathi Bhattacharyya., 2010. Evaluation of mathematical models describing drug release kinetics from theophylline S.R. floating matrix tablets J. Pharm Res, 3(9): 2265-2269.

Mutasem O. Taha., Wissam Nasser., Adel Ardakani., Hatim S. Alkhati., 2008. Sodium lauryl sulfate impedes drug release from zinc-crosslinked alginate beads: Switching from enteric coating release into biphasic profiles, Int. J. Pharm 350, 291-300.

Nagasamy Venkatesh D., Ramesh N., Karthick S., Mohammed Fakrudeen K., Uthayakumar B., Valliappan R.M., Vinu Deepak., Biplab Debnath., Samanta M. K., Suresh B., 2008. Design and *in vitro* evaluation of alginate beads of ambroxol hydrochloride. J. Pharm. Res., 1(2):139-142.

Olav Gaserod., Ian G. Jollife., Frank C.Hampson., Peter W. Dettmar., Gudmund Skjak-Braek., 1998. The enhancement of the bioadhesive properties of calcium alginate gel beads by coating with chitosan, Int. J. Pharm. 237-248.

Patil Basawaraj S., Rao Raghavendra NG., Jadhav suvarna., Kulkarni Upendra., Gada Mahesh., 2011. Estimation of candesartan cilexetil in bulk and tablet dosage form by UV spectrophotometric method, Int. J. Res. Ayur. pharm, 2011; 2(1): 204-206.

Patil D.A., Patil G.B., Deshmukh P.K., Belgamwar V.S., Fursule R.A., 2009. Chitosan coated mucoadhesive multiparticulate drug delivery system for gliclazide, Asian J. Pharm. and clin. Res, 2(2):62-68.

Patil J.S., Kamalapur M.V., Marapur S.C., Kadam D.V., 2010. Ionotropic gelation and polyelectrolyte complexation: the novel techniques to design hydrogel particulate sustained, modulated drug delivery system: A review, Digest J. Nanomaterials and Biostructures, 5(1): 241-248.

Paulo Costa and Jose Manuel Sousa Lobo., **2001.** Modeling and Comparison of dissolution profiles, Eur. J. Pharm sci, 123-133.

Payam Khazaeli., Abbas Pardakhty., and Fereshteh Hassanzadeh., 2008. Formulation of Ibuprofen Beads by Ionotropic Gelation Iranian J. Pharm. Res, 2008; 7 (3): 163-170.

Polona SMRDEL., Marija BOGATAJ., Ales MRHAR., 2008. The Influence of Selected Parameters on the Size and Shape of Alginate Beads Prepared by Ionotropic Gelation Sci Pharm. 76: 77–89.

Prabhakar Veera Reddy., Swathi Tedla., Sreenivas Reddy Banda., Suresh Bandari., Raju Jukanti., 2011. preparation and evaluation of mucoadhesive cefidinir microcapsules, J. Adv. Pharm. Tech. Res. 2(2): 115-122.

Rajput G.C., Dr. F.D.Majmudar, Dr. Patel J.K., Patel K.N., Thakor R.S., Patel B.P., Rajgor NB., 2010. Stomach Specific Mucoadhesive Tablets as Controlled Drug Delivery System – A Review Work, Int. J. Pharm & Bio Res. 1(1): 30-41.

Ravindra Reddy K and Sabitha Reddy P., 2010 Effect of different Co-polymers on Sodium Alginate Microcapsules Containing Isoniazid, Int. J. Pharm.Tech Res, 2010; 2(4): 2198-2203.

Raymond c. Rowe., paul j. Sheskey., Scan c. Owen., 2006. Hand book of pharmaceutical excipients, 5th edition, pharmaceutical press, London 159-162.

Rishi Pal., Anil P. S. Bhadoria., and Suman Ramteke., 2011. Preparation and characterization of sodium alginate-carbopol-934P based mucoadhesive microbeads, Der Pharm Lettre, 3(5) 1-11.

Sandhya Raj.S., Sundaramoorthy.K., Vetrichelvan.T., 2010. Formulation, development and *in-vitro* evaluation of valsartan mucoadhesive microcapsules, Int. J. Pharm and Res., 2(4): 1315-1327.

Sangeetha S., Sakthisaravanan V., Komala M., Harish G., Sivakumar V., 2010. Design and evaluation of gastroretentive beads of theophylline by ionotropic gelation, Int. J Pharm & Pharm. Sci, 2(3), 99-101.

Senthil Kumar K. L., Ashok kumar S., Ezhilmuthu R. P., 2010. Formulation and Evaluation of Didanosine Enteric Coated Sustained Release Tablet, J Biomed Sci and Res, 2 (3):126-131.

Simon bonita., 1983. A survey of microencapsulation process. Chapter 1 microencapsulation, method and industrial application" 2nd edition. Marcel dekker inc, Newyork,1982; 2-5.

Sivakumar R., Rajendran N., Narayanana N., 2011. Design of mucoadhesive hydrophilic beads entrapped with ketoprofen for delivery into small intestine, Res. J. Pharm., 2(3); 706-713.

Sunita Dahiya and Lalit Tyagi., 2008. Preparation and evaluation of oxytetracycline hydrochloride microbeads for delayed release, Pak. J Pharm., 21(2): 103-108.

Tavakol M., Vasheghani-Farahani E., Dolatabadi-Farahani T., Hashemi-Najafabadi S. 2010. Sulfasalazine release from alginate-N, O-carboxymethyl chitosan gel beads coated by chitosan, carbohydrates polym 326-330.

Veena Belgamwar., Viral Shah and Surana S.J., 2009. Formulation and Evaluation of Oral Mucoadhesive Multiparticulate System Containing Metoprolol Tartarate: An *In Vitro – Ex Vivo* Characterization, Cur. Drug Del, 6, 113-121.

www.astrazeneca.ca

www.drugs.com

www.fda.gov.in

www.rxlist.com